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Research Article

Synthesis, antioxidant and acidic properties of some new 3-alkyl(aryl)-4-(3-methoxy-4-cinnamoyloxy)benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones

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1,2,4-Triazol-5-one,
synthesis,
Schiff base,
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ABSTRACT

Nine new 3-alkyl(aryl)-4-(3-methoxy-4-cinnamoyloxy)benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**4**) were obtained by the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2**) with 3-methoxy-4-cinnamoyloxybenzaldehyde (**3**). The structures of nine new compounds were characterized from IR, ^{1}H-NMR, ^{13}C-NMR and UV spectral data. The synthesized compounds were analyzed for their *in vitro* antioxidant properties in three different methods. In addition, to determine the effects of solvents and molecular structure upon acidity compounds **4** were titrated potentiometrically with tetrabutylammonium hydroxide in four non-aqueous solvents, such as isopropyl alcohol, *tert*-butyl alcohol, acetone and *N,N*-dimethylformamide. HNP and pKa values changed depending on solvent and the substituents at C-3 in 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring.

TR

Bazı yeni 3-alkil(aril)-4-(3-metoksi-4-sinnamoiloksi)benzilidenamino-4,5-dihidro-1*H*-1,2,4-triazol-5-on bileşiklerinin sentezi, antioksidan ve asitlik özelliklerinin incelenmesi

Anahtar Kelimeler:
1,2,4-Triazol-5-on,
sentez,
Schiff bazı,
antioksidan aktivite,
asitlik

ÖZET

3-Alkil(aril)-4-amino-4,5-dihidro-1*H*-1,2,4-triazol-5-on (**2**) bileşiklerinin, 3-metoksi-4-sinnamoiloksibenzaldehid (**3**) ile reaksiyonları sonucunda dokuz yeni 3-alkil(aril)-4-(3-metoksi-4-sinnamoiloksi)-benzilidenamino-4,5-dihidro-1*H*-1,2,4-triazol-5-on (**4**) bileşikleri sentezlendi. Yeni dokuz adet bileşiğin yapısı IR, ^{1}H-NMR, ^{13}C-NMR ve UV spektrum verileri ile aydınlatıldı. Sentezlenen bileşiklerin *in vitro* antioksidan aktiviteleri üç farklı yöntemle analiz edildi. Ayrıca, çözücülerin ve moleküler yapının asitlik üzerine etkisini incelemek için, **4** bileşikleri dört farklı çözücüde (izopropil alkol, *tert*-butil alkol, aseton ve *N,N*-dimetilformamid) tetrabutilamonyum hidroksid ile potansiyometrik metotla titre edildi. HNP ve pKa değerlerinin çözücüye ve 4,5-dihidro-1*H*-1,2,4-triazol-5-on yapısında C-3 pozisyonundaki substutiente bağlı değiştiği gözlendi.

1. Introduction

Triazoles are five-membered heterocyclic compounds containing three nitrogen atoms. Some of the modern drugs containing a triazole moiety are triazolam, alprazolam, trazodone (antidepressant, anxiolytic), hexaconazole (antifungal), estazolam (hypnotic, sedative, tranquilizer), trapidil (hypotensive), etizolam (amnesic, anxiolytic, anticonvulsant, hypnotic, sedative and skeletal muscle relaxant), terconazole (antifungal), rilmazafon (hypnotic, anxiolytic) and rizatriptan (antimigrane agent) [1]. 1,2,4-Triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives have been found to present wide range of biological activities [2-9].

Moreover, 1,2,4-triazole and 4,5-dihydro-1*H*-1,2,4-triazol-5-one rings have weak acidic property. Therefore, some 1,2,4-triazoles and 4,5-dihydro-1*H*-1,2,4-triazol-5-ones were titrated potentiometrically with tetrabutylammonium hydroxide (TBAH) in non-aqueous solvents, and the p*Ka* values of the compounds were defined [8-10].

The role of reactive oxygen species (ROS) and of reactive nitrogen species (RNS) in food, drugs, and even living systems has gained an explosive interest in the last two decades. Hereby, scientists in various disciplines have been interested in naturally-occurring antioxidants and also related synthetic derivatives that could provide active components to avoid or minimize the effect of oxidative stress [11].

Exogenous chemicals and endogenous metabolic processes in the food system or in the human body might produce highly reactive free radicals, especially oxygen-derived radicals. They can be major mediators of damage to cell structures, including lipids and membranes, proteins, and nucleic acids, at high concentrations. Oxidative damages from ROS accumulates during the life cycle, and radical-related damage to biomolecules has been proposed to play an important role in the development of age-dependent diseases and other conditions [12].

In this study, because of a broad spectrum of applications to find their possible antioxidant activity, the newly synthesized 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were examined by using several methods including reducing power, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging and metal chelating

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activities. Furthermore, in order to define the p*Ka* values of the newly synthesized compounds, they were titrated potentiometrically with TBAH in four non-aqueous solvents; isopropyl alcohol, *tert*-butyl alcohol, acetone and *N,N*-dimethylformamide (DMF).

2. Materials and methods

Chemical reagents used in this study were bought from Merck AG, Aldrich and Fluka. Melting points were taken using a WRS-2A Microprocessor Melting Point Apparatus in an open capillary tube and were uncorrected. The infrared spectra were recorded on a Bruker Alpha FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were defined in deuterated dimethyl sulfoxide with TMS as internal standard using a Bruker Ultrashield Plus Biospin spectrophotometer at 400 MHz and 100 MHz, respectively. UV absorption spectra were evaluated in 10 mm quartz cells between 200 and 400 nm using a PG Instruments Ltd T80 UV/Vis spectrometer. Extinction coefficients (ε) were clarified in L·mol^{-1}·cm^{-1}.

2.1. General procedure for the synthesis of compounds 4

3-Methoxy-4-hydroxybenzaldehyde (0.01 mol) dissolved in ethyl acetate (100 mL) was treated with cinnamoyl chloride (0.01 mol), and to the solution was slowly added triethylamine (0.01 mol) with stirring at 0-5 °C. Stirring was continued for 2 h, and then the mixture was refluxed for 3 h and filtered. The filtrate was evaporated *in vacuo*, and the crude product was washed with water and recrystallized from ethanol to obtain compound **3** [13]: Yield 98.0%, mp 94 °C; IR (KBr) υ (cm^{-1}): 3058 (C=CH), 2801 and 2737 (CHO), 1728, 1693 (C=O), 1628 (C=C), 1265 (COO), 761 and 712 (monosubstitue benzenoid ring). UV λ_{max} (ε): 280 (17431), 260 (18442), 224 (19758). Then the corresponding compound **2** (0.01 mol) was dissolved in acetic acid (20 mL) and treated with 3-methoxy-4-cinnamoyloxybenzaldehyde 3 (0.01 mol). The mixture was refluxed for 1.5 h and then evaporated at 50-55 °C *in vacuo.* Several recrystallizations of the residue from ethanol gave pure compounds 3-alkyl(aryl)-4-(3-methoxy-4-cinnamoyloxy)benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**4**) as colorless crystals.

2.1.1. 3-Methyl-4-(3-methoxy-4-cinnamoyloxy benzylideneamino-4,5-dihydro-1H-1,2,4-triazol-5-one (4a)

Yield 97.9%, m.p. 181 °C; IR (KBr) υ (cm^{-1}): 3160 (NH), 3037 (C=CH), 1726, 1692 (C=O), 1628 (C=C), 1595 (C=N), 1264 (COO), 765 and 684 (monosubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d$_6$), δ (ppm): 2.31 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 6.91 (d, 1H, *J* = 16.00 Hz, CH=CH), Arom. H: [7.32 (d, 1H, *J* = 8.00 Hz), 7.47-7.51 (m, 4H), 7.62 (s, 1H), 7.81-7.84 (m, 2H)], 7.88 (d, 1H, *J* = 16.00 Hz, CH=CH), 9.74 (s, 1H, N=CH), 11.84 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$), δ (ppm): 11.58 (CH_3), 56.43 (OCH_3), Arom. C: [111.92, 117.13, 121.00, 124.06, 129.20 (2C), 129.50 (2C), 131.46, 132.98, 134.28, 142.20, 147.32], 151.72 (Arom. C + CH=CH), 144.80 (Triazol-C_3), 151.87 (N=CH), 153.45 (Triazol-C_5), 164.60 (COO). UV λ_{max} (ε): 296 (24880), 284 (25172), 270 (23703), 224 (30683).

2.1.2. 3-Ethyl-4-(3-methoxy-4-cinnamoyloxy) benzylideneamino-4,5-dihydro-1H-1,2,4-triazol-5-one (4b)

Yield 97.9%, m.p. 170 °C; IR (KBr) υ (cm^{-1}): 3168 (NH), 3044 (C=CH), 1715, 1702 (C=O), 1633 (C=C), 1584 (C=N), 1269 (COO), 763 and 704 (monosubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d$_6$), δ (ppm): 1.23 (t, 3H, *J* = 7.60 Hz, CH_2CH_3), 2.72 (q, 2H, *J* = 7.60 Hz, CH_2CH_3), 3.86 (s, 3H, OCH_3), 6.92 (d, 1H, *J* = 16.00 Hz, CH=CH), Arom. H: [7.32 (d, 1H, *J* = 8.40 Hz), 7.47-7.51 (m, 4H), 7.62 (s, 1H), 7.82-7.84 (m, 2H)], 7.89 (d, 1H, *J* = 16.00 Hz, CH=CH), 9.74 (s, 1H, N=CH), 11.87 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$), δ (ppm): 10.49 (CH_2CH_3), 18.99 (CH_2CH_3), 56.42 (OCH_3), Arom. C: [117.13, 120.87, 124.09, 129.20 (2C), 129.49 (2C), 131.46, 133.02, 134.29, 142.19, 147.33], 151.86 (Arom. C + CH=CH), 148.56 (Triazol-C_3), 151.86 (N=CH), 153.43 (Triazol-C_5), 164.61 (COO). UV λ_{max} (ε): 304 (22066), 296 (23005), 286 (23038), 268 (22956), 226 (21756).

2.1.3. 3-n-Propyl-4-(3-methoxy-4-cinnamoyloxy) benzylideneamino-4,5-dihydro-1H-1,2,4-triazol-5-one (4c)

Yield 99.5%, m.p. 163 °C; IR (KBr) υ (cm^{-1}): 3165 (NH), 3032 (C=CH), 1734, 1696 (C=O), 1626 (C=C), 1580 (C=N), 1266 (COO), 763 and 681 (monosubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d$_6$), δ (ppm): 0.97 (t, 3H, *J* = 7.60 Hz, $CH_2CH_2CH_3$), 1.71 (sext, 2H, *J* = 7.60 Hz, $CH_2CH_2CH_3$), 2.67 (t, 2H, *J* = 7.60 Hz, $CH_2CH_2CH_3$), 3.86 (s, 3H, OCH_3), 6.92 (dd, 1H, *J* = 16.00, 2.40 Hz, CH=CH), Arom. H: [7.33 (d, 1H, *J* = 8.40 Hz), 7.46-7.51 (m, 4H), 7.61 (s, 1H), 7.82-7.84 (m, 2H)], 7.89 (d, 1H, *J* = 16.00 Hz, CH=CH), 9.74 (s, 1H, N=CH), 11.89 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$), δ (ppm): 13.98 ($CH_2CH_2CH_3$), 19.46 ($CH_2CH_2CH_3$), 27.19 ($CH_2CH_2CH_3$), 56.39 (OCH_3), Arom. C: [112.00, 117.12, 120.81, 124.10, 129.20 (2C), 129.49 (2C), 131.45, 133.03, 134.28, 142.18, 147.33], 151.79 (Arom. C + CH=CH), 147.44 (Triazol-C_3), 151.87 (N=CH), 153.39 (Triazol-C_5), 164.61 (COO). UV λ_{max} (ε): 296 (27639), 290 (27903), 224 (24746).

2.1.4. 3-Benzyl-4-(3methoxy-4-cinnamoyloxy) benzylideneamino-4,5-dihydro-1H-1,2,4-triazol-5-one (4d)

Yield 95.7%, m.p. 158 °C; IR (KBr) υ (cm^{-1}): 3163 (NH), 3026 (C=CH), 1724, 1704 (C=O), 1633 (C=C), 1594 (C=N), 1265 (COO), 762 and 703 (monosubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d$_6$), δ (ppm): 3.86 (s, 3H, OCH_3), 4.09 (s, 2H, CH_2Ph), 6.92 (dd, 1H, *J* = 16.00, 1.20 Hz, CH=CH), Arom. H: [7.23-7.65 (m, 9H), 7.82-7.83 (m, 2H), 7.87-7.90 (m, 2H)], 7.91 (d, 1H, *J* = 15.60 Hz, CH=CH), 9.72 (s, 1H, N=CH), 12.01 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$), δ (ppm): 31.71 (CH_2Ph), 56.39 (OCH_3), Arom. C: [112.41, 117.13, 121.54, 124.11, 127.21, 128.96 (2C), 129.20 (2C), 129.24 (2C), 129.49 (2C), 131.52, 132.97, 134.28, 135.61, 142.24, 147.33], 151.73 (Arom. C + CH=CH), 146.71 (Triazol-C_3), 152.18 (N=CH), 152.83 (Triazol-C_5), 164.61 (COO). UV λ_{max} (ε): 296 (24419), 292 (24542), 284 (24655), 268 (24218), 224 (22516).

2.1.5. 3-p-Methylbenzyl-4-(3methoxy-4-cinnamoyloxy) benzylideneamino-4,5-dihydro-1H-1,2,4-triazol-5-one (4e)

Yield 96.9%, m.p. 176 °C; IR (KBr) υ (cm^{-1}): 3133 (NH), 3072 (C=CH), 1734, 1701 (C=O), 1641 (C=C), 1605 (C=N), 1265 (COO), 822 (1,4-disubstituted benzenoid ring), 758 and 707 (monosubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d$_6$), δ (ppm): 2.25 (s, 3H, $PhCH_3$), 3.86 (s, 3H, OCH_3), 4.03 (s, 2H, CH_2Ph), 6.92 (d, 1H, *J* = 16.00 Hz, CH=CH), Arom. H: [7.12 (d, 2H, *J* = 7.60 Hz), 7.23 (d, 2H, *J* = 7.60 Hz), 7.31 (d, 1H, *J* = 8.00 Hz), 7.43 (dd, 1H, *J* = 8.00, 1.60 Hz), 7.47-7.49 (m, 3H), 7.54 (d, 1H, *J* = 1.60 Hz), 7.82-7.85 (m, 2H)], 7.89 (d, 1H, *J* = 16.00 Hz, CH=CH), 9.69 (s, 1H, N=CH), 12.01 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$), δ (ppm): 21.07 ($PhCH_3$), 31.31 (CH_2Ph), 56.38 (OCH_3), Arom. C: [111.12, 117.12, 121.57, 124.05, 129.07 (2C), 129.21 (2C), 129.50 (2C), 129.53 (2C), 131.47, 132.98, 133.26, 134.28, 136.28, 142.22, 147.35], 151.71 (Arom. C + CH=CH), 146.87 (Triazol-C_3), 151.84 (N=CH), 152.78 (Triazol-C_5), 164.62 (COO). UV λ_{max} (ε): 296 (31918), 288 (32187), 224 (28712).

2.1.6. 3-p-Methoxybenzyl-4-(3methoxy-4-cinnamoyloxy) benzylideneamino-4,5-dihydro-1H-1,2,4-triazol-5-one (4f)

Yield 97.4%, m.p. 185 °C; IR (KBr) υ (cm^{-1}): 3172 (NH), 3050 (C=CH), 1733, 1690 (C=O), 1637 (C=C), 1580 (C=N), 1245 (COO), 810 (1,4-disubstituted benzenoid ring), 759 and 682 (monosubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d$_6$), δ (ppm): 3.71 (s, 3H, OCH_3 -p), 3.87 (s, 3H, OCH_3), 4.01 (s, 2H, CH_2Ph), 6.92 (d, 1H, *J* = 16.00 Hz, CH=CH), Arom. H: [7.27 (d, 2H, *J* = 8.00 Hz), 7.31 (d, 1H, *J* = 8.00 Hz), 7.44 (dd, 1H, *J* = 8.00, 2.00 Hz), 7.47-7.48 (m, 3H), 7.56 (d, 1H, *J* = 2.00 Hz), 7.82-7.84 (m, 2H)], 7.89 (d, 1H,

J = 16.00 Hz, CH=CH), 9.71 (s, 1H, N=CH), 12.00 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 30.84 (CH_2Ph), 55.48 (OCH_3 -p), 56.41 (OCH_3), Arom. C: [111.24, 114.37 (2C), 117.13, 121.50, 124.05, 128.13, 129.20 (2C), 129.49 (2C), 130.27 (2C), 131.50, 133.00, 134.28, 142.24, 147.33, 151.74], 158.00 (Arom. C + CH=CH), 147.03 (Triazol-C_3), 151.87 (N=CH), 152.86 (Triazol-C_5), 164.60 (COO). UV λ_{max} (ε): 300 (25885), 296 (27206), 288 (27397), 226 (25623).

2.1.7. 3-p-Chlorobenzyl-4-(3methoxy-4-cinnamoyloxy) benzylideneamino-4,5-dihydro-1H-1,2,4-triazol-5-one (4g)

Yield 98.7%, m.p. 177 °C; IR (KBr) υ (cm^{-1}): 3162 (NH), 3024 (C=CH), 1723, 1694 (C=O), 1633 (C=C), 1595 (C=N), 1283 (COO), 848 (1,4-disubstituted benzenoid ring), 762 and 681 (monosubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.85 (s, 3H, OCH_3), 4.10 (s, 2H, CH_2Ph), 6.93 (d, 1H, *J* = 16.00 Hz, CH=CH), Arom. H: [7.31 (d, 1H, *J* = 8.40 Hz), 7.38-7.42 (m, 4H), 7.43 (dd, 1H, *J* = 8.00, 1.60 Hz), 7.45-7.49 (m, 3H), 7.52 (d, 1H, *J* = 2.00 Hz), 7.82-7.85 (m, 2H)], 7.88 (d, 1H, *J* = 16.00 Hz, CH=CH), 9.70 (s, 1H, N=CH), 12.04 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 30.99 (CH_2Ph), 56.42 (OCH_3), Arom. C: [111.23, 117.12, 121.55, 124.06, 128.90 (2C), 129.21 (2C), 129.50 (2C), 131.13 (2C), 131.47, 131.90, 132.91, 134.28, 135.38, 142.26, 147.35], 151.69 (Arom. C + CH=CH), 146.38 (Triazol-C_3), 151.86 (N=CH), 153.02 (Triazol-C_5), 164.60 (COO). UV λ_{max} (ε): 296 (26613), 288 (27490), 224 (26113).

2.1.8. 3-m-Chlorobenzyl-4-(3methoxy-4-cinnamoyloxy) benzylideneamino-4,5-dihydro-1H-1,2,4-triazol-5-one (4h)

Yield 98.7%, m.p. 179 °C; IR (KBr) υ (cm^{-1}): 3167 (NH), 3054 (C=CH), 1734, 1698 (C=O), 1634 (C=C), 1574 (C=N), 1269 (COO), 855 and 815 (1,3-disubstituted benzenoid ring), 762 and 681 (monosubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.87 (s, 3H, OCH_3), 4.13 (s, 2H, CH_2Ph), 6.92 (d, 1H, *J* = 16.00 Hz, CH=CH), Arom. H: [7.31 (d, 1H, *J* = 8.00 Hz), 7.31-7.38 (m, 3H), 7.43 (dd, 1H, *J* = 8.00, 1.60 Hz), 7.46-7.49 (m, 4H), 7.56 (d, 1H, *J* = 2.00 Hz), 7.82-7.87 (m, 2H)], 7.88 (d, 1H, *J* = 16.40 Hz, CH=CH), 9.71 (s, 1H, N=CH), 12.06 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 31.23 (CH_2Ph), 56.42 (OCH_3), Arom. C: [111.12, 117.12, 121.65, 124.04, 127.26, 127.98, 129.21 (2C), 129.26, 129.52 (2C), 130.80, 131.47, 132.90, 133.46, 134.28, 138.82, 142.29, 147.35], 151.67 (Arom. C + CH=CH), 146.21 (Triazol-C_3), 151.88 (N=CH), 153.00 (Triazol-C_5), 164.60 (COO). UV λ_{max} (ε): 294 (25148), 288 (25233), 224 (22602).

2.1.9. 3-Phenyl-4-(3methoxy-4-cinnamoyloxy) benzylideneamino-4,5-dihydro-1H-1,2,4-triazol-5-one (4i)

Yield 97.1%, m.p. 171 °C; IR (KBr) υ (cm^{-1}): 3159 (NH), 3057 (C=CH), 1735, 1687 (C=O), 1635 (C=C), 1603 (C=N), 1262 (COO), 761 and 689 (monosubstituted benzenoid ring). ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.84 (s, 3H, OCH_3), 6.93 (d, 1H, *J* = 16.00 Hz, CH=CH), Arom. H: [7.34 (d, 1H, *J* = 8.00 Hz), 7.46-7.49 (m, 4H), 7.53-7.58 (m, 3H), 7.61 (d, 1H), 7.82-7.85 (m, 2H), 7.93-7.95 (m, 2H)], 7.89 (d, 1H, *J* = 16.00 Hz, CH=CH), 9.70 (s, 1H, N=CH), 12.42 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 56.33 (OCH_3), Arom. C: [111.79, 117.10, 121.38, 124.19, 127.07, 128.50 (2C), 129.00 (2C), 129.22 (2C), 129.50 (2C), 130.65, 131.48, 132.84, 134.27, 142.38, 147.38], 151.82 (Arom. C + CH=CH), 145.06 (Triazol-C_3), 151.89 (N=CH), 155.85 (Triazol-C_5), 164.62 (COO). UV λ_{max} (ε): 294 (27430), 284 (28810), 224 (25850).

2.2.1. Chemicals antioxidant activity

Butylated hydroxytoluene (BHT) was acquired from E. Merck. Ferrous chloride, 1,1-diphenyl-2-picryl-hydrazyl (DPPH·), 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine (ferrozine), *α*-tocopherol, trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA) and butylated hydroxyanisole (BHA) were acquired from Sigma–Aldrich.

2.2.2. Reducing power

The reducing power of the newly synthesized compounds was measured by the method of Oyaizu [14] as expressed in the literature [8,9].

2.2.3. Free radical scavenging activity

Free radical scavenging activity of the newly synthesized compounds was estimated by DPPH·, using the method of Blois [15] as expressed in the literature [8,9].

2.2.4. Metal chelating activity

The chelation of ferrous ions by the newly synthesized compounds and standards were determined according to the method of Dinis et al. [16] as expressed in the literature [8,9].

2.3. Potentiometric titrations

A Jenco model ion analyzer and an Ingold pH electrode were used for potentiometric titrations. For every compound that was titrated, the 0.001 M solution was separately prepared in each non-aqueous solvent. The 0.05 M solution of TBAH in isopropyl alcohol, which is properly used in the titration of acids, was used as titrant. The mV values provided in pH-meter were recorded. Eventually, the half-neutralization potential (HNP) values were determined by drawing the mL (TBAH)-mV graphs.

3. Results and discussion

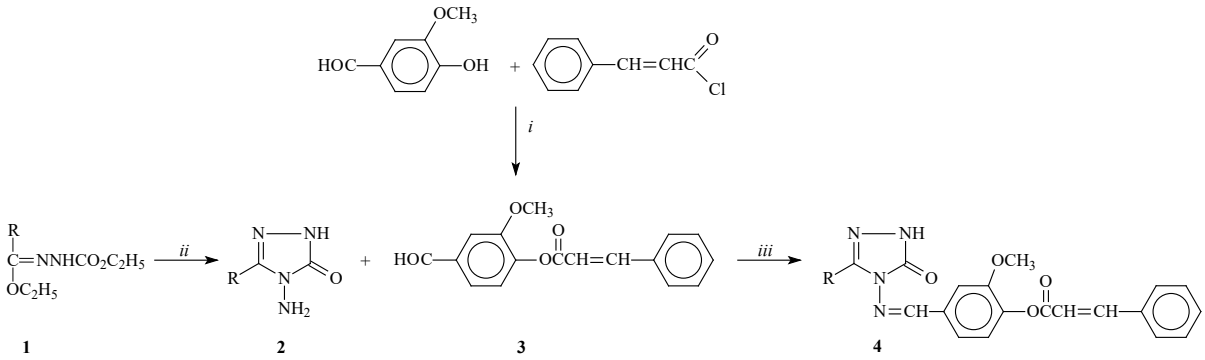
The 3-alkyl(aryl)-4-(3-methoxy-4-cinnamoyloxy)benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **4a-i** were obtained. The starting compounds 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones **2a-i** were obtained from the reactions of the corresponding ester ethoxycarbonylhydrazones **1a-i** with an aqueous solution of hydrazine hydrate as explained in the literature [17,18]. Compounds **4** were prepared from the reactions of compounds **2** with 3-methoxy-4-cinnamoyloxybenzaldehyde **3** which were synthesized by the reactions of 3-methoxy-4-hydroxybenzaldehyde with cinnamoyl chloride by using triethylamine (Fig. 1). The structures of nine new **4** type compounds were identified with IR, ^{1}H NMR, ^{13}C NMR and UV spectral data.

3.1. Antioxidant activity

The antioxidant activities of nine newly synthesized compounds **4a-i** were determined. Several methods have been used to define antioxidant properties and the methods used in the study are explained below:

3.1.1. Total reductive capability using the potassium ferricyanide reduction method

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



i) Et_3N, AcOEt, 0-5°C; *ii*) $N_2H_4.H_2O$, reflux, 6 h; *iii*) AcOH, reflux, 1 h
a) R = CH_3, b) R = CH_2CH_3, c) R = $CH_2CH_2CH_3$, d) R = $CH_2C_6H_5$, e) R = $CH_2C_6H_4CH_3$ (*p*-),
f) R = $CH_2C_6H_4OCH_3$ (*p*-), g) R = $CH_2C_6H_4Cl$ (*p*-), h) R = $CH_2C_6H_4Cl$ (*m*-), i) R = C_6H_5

Fig. 1. Synthesis route of compounds **2 – 4**

3.1.2. DPPH· radical scavenging activity

The model of scavenging stable DPPH radical is a widely used method to investigate antioxidant properties in a relatively short time compared with the other methods. The effect of antioxidants on DPPH radical scavenging was thought to be because of their hydrogen donating ability [21]. DPPH is a stable free radical and accepts hydrogen radical or an electron to become a stable diamagnetic molecule [22]. The reduction capability of DPPH radicals was established by decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of DPPH radical was caused by antioxidants due to the reaction between antioxidant molecules and radical progress, which resulted in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Therefore, DPPH. is usually used as a substrate to evaluate anti-oxidative activity of antioxidants [23]. In this paper, antiradical activities of the newly synthesized compounds and standard antioxidant compounds including BHA, BHT and α-tocopherol were determined by using DPPH· method. Finally, all of the amounts of the compounds showed lower absorbance change then blank. Hereby, no activities were observed to reduce metal ions complexes to their lower oxidation state or to take part in any electron transfer reaction. In other words, compounds showed no radical scavenging activities.

3.1.3. Ferrous ion chelating activity

The chelating effect towards ferrous ions by the newly synthesized compounds and standard compounds was detected. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Measurement of color reduction allows estimation of the chelating activity of the coexisting chelator [24]. Transition metals have an important role in the generation oxygen free radicals in living organisms. The ferric iron (Fe^{3+}) is the relatively biologically inactive form of iron. However, it can be reduced to the active Fe^{2+}, depending on condition, particularly pH [25] and oxidized back through Fenton type reactions with the production of hydroxyl radical or Haber-Weiss reactions with superoxide anions. The production of these radicals may lead to protein modification, lipid peroxidation and DNA damage. Chelating agents may not activate metal ions and potentially inhibit the metal-dependent processes [26]. Additionally, the production of highly active ROS, for example, $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$ is also catalyzed by free iron though Haber-Weiss reactions:

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

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

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

Fe^{3+} ion also produces radicals from peroxides, even though the rate is tenfold less than that of Fe^{2+} ion, which is the most powerful pro-oxidant among the diverse types of metal ions [27]. Ferrous ion chelating activities of the newly synthesized compounds **4**, EDTA and α-tocopherol are given in Fig. 1. Low absorbance at 562 nm demonstrates high metal chelating activity. The data obtained from Fig.1 reveal that the metal chelating effects of the newly synthesized compounds **4** were not concentration-dependent in the concentration range studied. Nevertheless, the compounds **4** indicate a marked capacity for iron binding at the lowest concentration, proposing that their action as peroxidation protectors may be related to their iron binding capacity. The metal chelating effect of the compounds and references decreased in order of EDTA > 4a > 4f > 4b > 4e > 4c > 4i > 4h > 4d > α-tocopherol > 4g, which were 82.5, 65.8, 65.5, 64.7, 62.9, 61.8, 60.0, 59.6, 56.0, 55.6, 54.9 (%), at the lowest concentration, respectively.

3.2. Potentiometric titrations

In order to detect the p*Ka* values of the newly synthesized compounds **4**, they were titrated potentiometrically with TBAH in four non-aqueous solvents. The mV values read in each titration were plotted against 0.05 M TBAH volumes (mL) added, and potentiometric titration curves were provided for all the cases. From the titration curves, the HNP values were measured, then the corresponding p*Ka* values were calculated. The data obtained from the potentiometric titrations were interpreted, and the effect of the C-3 substituent in 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring as well as solvent effects were evaluated.

As an example, the potentiometric titration curves for 0.001 M solutions of compound **4b** titrated with 0.05 M TBAH in the solvents are given in the Fig. 3.

When the dielectric permittivity of the solvents is taken into consideration, the acidity order can be given as follows: DMF (ε=36.7) > acetone (ε=36) > isopropyl alcohol (ε=19.4) > *tert*-butyl alcohol (ε=12). As shown in Table, the acidity order of the solvents for the compound **4a** is: Acetone > DMF > *tert*-butyl alcohol, for the compound **4c** it is: DMF > isopropyl alcohol > acetone > tert-butyl alcohol, for the compound **4d** it is: Acetone > isopropyl alcohol >

DMF, for the compound **4f** it is: isopropyl alcohol > *tert*-butyl alcohol, for the compound **4g** it is: isopropyl alcohol > tert-butyl alcohol > DMF > acetone, for the compound **4h** it is: isopropyl alcohol > acetone > tert-butyl alcohol, while the order for the compound **4i** is: tert-butyl alcohol > isopropyl alcohol. Moreover, for the compound **4a** in isopropyl alcohol; for the compound **4b** in DMF, *tert*-butyl alcohol, isopropyl alcohol; for the compound **4d** in *tert*-butyl alcohol; for the compound **4e** in DMF, acetone, *tert*-butyl alcohol; for the compounds **4f** and **4i** in DMF, acetone and for the compound **4h** in DMF, the HNP values and the corresponding p*Ka* values were not acquired.

The acidity of a compound depends on some factors, as known. The two most important ones are the molecular structure and solvent effect [8-10]. Fig. 3 and Table show that the HNP values and corresponding p*Ka* values acquired from the potentiometric titrations depend on the used non-aqueous solvents and the substituents at C-3, in 4,5-dihydro-1*H*-1,2,4-triazol-5-one ring.

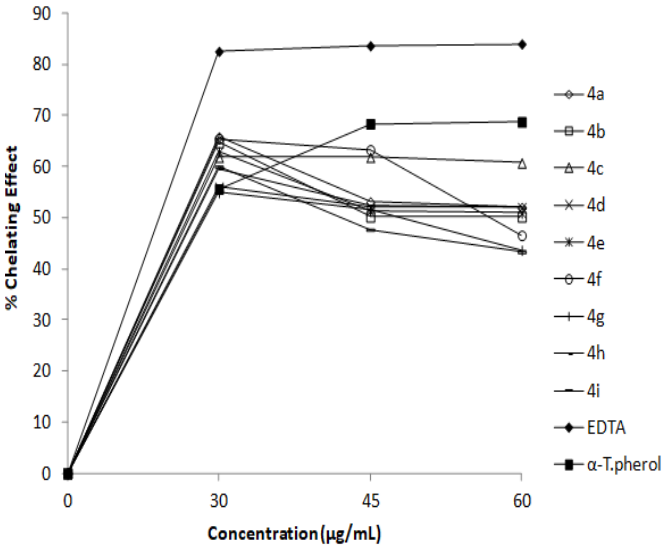


Fig. 2. Metal chelating effect of different amounts of the compounds **4a-i,** EDTA and *α*-tocopherol on ferrous ions

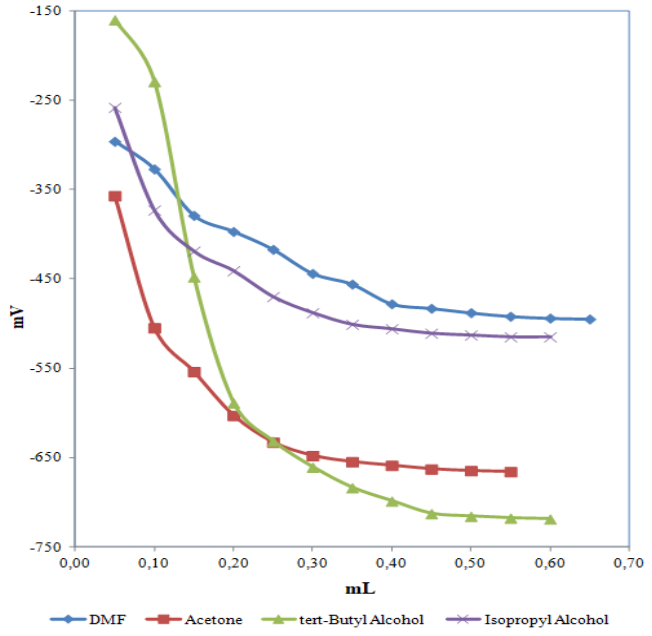


Fig. 3. Potentiometric titration curves of 0.001 M solutions of compound **4b** titrated with 0.05 M TBAH in DMF, acetone, *tert*-butyl alcohol and isopropyl alcohol at 25 °C.

Table. The HNP and the corresponding p*Ka* values of compounds **4a-i** in DMF, acetone, *tert*-butyl alcohol and isopropyl alcohol.

Comp.	Isopropyl alcohol		*tert*-Butyl alcohol		DMF		Acetone	
	HNP (mV)	pK_a	HNP (mV)	pK_a	HNP (mV)	pK_a	HNP (mV)	pK_a
4a	-	-	-339	14.15	-289	13.95	-257	13.05
4b	-	-	-	-	-	-	-233	12.52
4c	-279	13.27	-414	16.85	-278	13.29	-304	14.14
4d	-299	10.58	-	-	-357	15.42	-190	11.52
4e	-262	13.00	-	-	-	-	-	-
4f	-377	15.96	-598	-	-	-	-	-
4g	-350	15.96	-365	14.97	-382	16.53	-383	15.33
4h	-288	13.24	-430	17.28	-	-	-384	15.89
4i	-296	13.95	-285	13.54	-	-	-	-

4. Conclusions

The synthesis, acidic properties and *in vitro* antioxidant evaluation of newly synthesized 4,5-dihydro-1*H*-1,2,4-triazol-5-ones were achieved. Although the antioxidant properties of the compounds were not very bright, determination of p*Ka* values of the active constituent of certain pharmaceutical preparations is also important due to the transport behavior, distribution and bonding to receptors.

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