Bioisosteric modification on melatonin: synthesis of new naphthalene derivatives, in vitro antioxidant activity and cytotoxicity studies

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Melatonin (MLT) is a strong free radical scavenger that protects the body from the deleterious effects of excess oxidants. Synthesis of MLT analogue compounds with antioxidant potency has recently attracted the interest of researchers. In general, the strategy consists of modifying the groups in the different sites of the indole ring or replacing the indole ring with an analogue. As part of our ongoing research, the antioxidant capacity and cytotoxicity of newly synthesized MLT analogue naphthalene derivatives were evaluated. The radical scavenging activity was tested by a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Most of the synthesized compounds showed significant antioxidant activity in comparison to MLT. The structure-activity relationship was identified. The in vitro cytotoxic effects of the synthesized compounds were also investigated in CHO-K1 cells using the MTT assay.

Keywords: Antioxidant. Cytotoxic. Melatonin. MTT. DPPH.

INTRODUCTION

Melatonin (MLT) is a neurohormone secreted by the pineal gland in the human body (Maharaj et al., 2005). Biosynthesis of MLT (N-acetyl-5-methoxytryptamine) starts with the amino acid L-tryptophan which is a precursor amino acid for synthesizing both serotonin and MLT in the pineal gland (Yonei et al., 2010). MLT has been recognized as a specific hormone of the pineal gland, but it is also secreted in the gastrointestinal tract, skin, retina, brain and some other parts of the body (Reiter et al., 2000; Suzuki et al., 2008). MLT and its derivatives are responsible of the regulation of the circadian rhythm. In addition to this important function, MLT performs numerous tasks in the body such as oncostatic effects, immune modulation and antioxidant activities (Danilov, Kurganova, 2016). Overproduction of reactive oxygen species (ROS) can cause oxidative stress (OS), which may lead to vital damage to cell structures such as proteins and DNA (Valko et al., 2007; Yamashita et al., 2013). MLT and its metabolites have noteworthy antioxidant properties and are able to function as endogenous free-radical scavengers (Reiter et al., 1999; Shirinzadeh et al., 2016). According to recent research, MLT has protective effects in some of OS-related diseases such as Huntington’s disease, Parkinson’s disease, Alzheimer’s disease and ageing (Suzen, 2007; Allegra et al., 2003; Suzen et al., 2013; Carocci, Catalano, Sinicropi, 2014).

Long-term studies on the MLT molecule brought about only a few commercialized MLT analogues such as Circadin, ramelteon, agomelatine and Tasimelteon (Lemoine, Zisapel, 2012; Hirai et al., 2005). Substitution of the indole ring of MLT with other rings which are isosteres for indole was of major interest. One of the isostere rings is naphthalene (Landagaray et al., 2016). Agomelatine is the first naphthalenic analogue of MLT that was confirmed as an antidepressant agent for treatment of major depressive disorders by the European Medicines Agency (Ettaoussi et al., 2012). Many studies have reported the antioxidant properties of
agomelatine (Aguiar et al., 2013). As part of our ongoing research, naphthalene derivatives analogous to MLT were synthesized (De Mello et al., 2016; Azim, Agarwal, Vohora, 2017). In general, the strategy in this study consisted of replacing the indole ring with the bioisosteric naphthalene ring. All twenty-one compounds except 1h (Robev, 1981), 1r (Robev, 1968) and 1u (Weil, Ostermeier, 1921; Ding et al., 2017) were new. Furthermore, the compounds 1m, 1p and 1o have CAS registry numbers, but there is no information in the literature related to the biological activity of these. The synthesized compounds were characterized on the basis of 1H and 13C NMR, mass spectra and elemental analyses. The biological activity of the compounds was investigated in in vitro conditions by a well-known 1,1-diphenyl 2-picrylhydrazyl (DPPH) radical scavenging activity assay. The in vitro cytotoxic effects of the synthesized compounds were investigated in CHO-K1 cells using the MTT assay.

**MATERIAL AND METHODS**

This study was designed to synthesize, characterize and investigate the potential antioxidant and cytotoxic effects of new MLT analogue compounds containing the naphthalene ring instead of the indole ring. Two major modifications may be seen on MLT. The first one was the replacement of the indole ring of MLT with naphthalene which is bioisosteric with indole. The second modification was made on the acetylamino-ethyl side chain by formation of imine (Figure 1). Two series of new compounds were synthesized (Table I). It is known that there are different opinions about the antioxidant efficacy of the methoxy group in the MLT molecule (Shirinzadeh et al., 2010; Spadoni et al., 2006; Letra-Vilela et al., 2016). To identify the antioxidant role of the methoxy group on the naphthalene ring, 6-methoxy naphthalene and non-methoxy naphthalene series were synthesized.

**Chemistry – experimental**

Uncorrected melting points were determined with a Stuart melting point SMP30 apparatus. The 1H and 13C NMR spectra were measured with a Varian 400 MHz spectrometer device (Palo Alto, CA) using TMS as an internal standard and DMSO-d_6 as solvent. ESI mass spectra were determined by a Waters Micromass ZQ device. Elemental analyses were performed using a CHNS-932 instrument (Leco Corporation, St. Joseph,

**FIGURE 1** - Modifications on MLT molecule to develop new analogues.
MI). All spectral analyses were performed at the Central Laboratory of the Faculty of Pharmacy at Ankara University. Chromatography was carried out using Merck silica gel 60 (230–400 mesh ASTM). The chemical reagents that were used in synthesis were purchased from Sigma (Germany) and Aldrich (USA).

**Chemistry**

The designed compound was synthesized by condensation of phenylhydrazine with 6-methoxy-2-naphthaldehyde or 2-naphthaldehyde. All new imines were obtained by using a methodology similar to that in a previous study (Kidwai, Negi, Gupta, 1994). The synthesized compounds were characterized on the basis of $^1$H and $^{13}$C NMR, mass spectra and elemental analyses.

**General procedure for the synthesis of compounds 1a-u**

1 mmol of 6-methoxy-2-naphthaldehyde and 1.3 mmol of phenyl hydrazine hydrochloride or its derivatives were dissolved in absolute ethanol (20 mL) and heated for 60 min on a hot water bath in the presence of CH$_3$COONa (0.4 g). After completion of the reaction, the reaction mixture was cooled to room temperature. The precipitate was collected, washed with cold EtOH and recrystallized from EtOH to achieve 1a–u with 60 to 94% yield.

1-(2-bromophenyl)-2-((6-methoxynaphthalen-2-yl)methylene)hydrazine (1a)

Yield 94.63%, m.p. 148 °C; $^1$H-NMR: δ 3.93 (s, 3H, OCH$_3$); 6.73-8.00 (m, 10H ArH); 7.81 (s, 1H, NH); 7.94 (s, 1H, HC=N); $^{13}$C-NMR: δ 55.35 (OCH$_3$), 106.15, 106.84, 114.58, 119.14, 120.53, 123.59, 127.02, 127.35, 128.59, 128.72, 129.67, 130.50, 132.26, 135.05, 140.13, 141.57 (HC=N), 158.24: MSI MS m/z 355 (M+, 90 %), 357 (M+2, 100 %). Anal. calcd. for C$_{18}$H$_{15}$BrN$_2$O: C, 60.86%; H, 4.26%; N, 7.89%. Found: C, 60.76%; H, 4.78%; N, 7.61%.

1-(3-bromophenyl)-2-((6-methoxynaphthalen-2-yl)methylene)hydrazine (1b)

Yield 86.77%, m.p. 205 °C; $^1$H-NMR: δ 3.91 (s, 3H, OCH$_3$); 6.86-7.99 (m, 10H ArH); 7.98 (s, 1H, OCH$_3$); 7.81 (s, 1H, NH); 7.92 (s, 1H, HC=N); $^{13}$C-NMR: δ 55.52 (OCH$_3$), 106.15, 106.84, 114.58, 119.14, 120.53, 123.59, 127.02, 127.35, 128.59, 128.72, 129.67, 130.50, 132.26, 135.05, 140.13, 141.57 (HC=N), 158.24: MSI MS m/z 357 (M+, 90 %), 359 (M+2, 100 %). Anal. calcd. for C$_{19}$H$_{16}$BrN$_2$O: C, 60.46%; H, 4.26%; N, 7.89%. Found: C, 60.76%; H, 4.78%; N, 7.61%.
1-(4-chlorophenyl)-2-((6-methoxynaphthalen-2-yl)methylene)hydrazine (1f)

Yield 90.68%, m.p. 229 °C; 1H-NMR: δ 3.86 (s, 3H, O-CH₃); 7.07-7.92 (m, 10H Ar-H); 7.97 (s, 1H, HC=N); 10.46 (s, 1H, NH); 13C-NMR: δ 55.21 (OCH₃); 106.32, 113.34, 118.86, 121.79, 123.01, 126.00, 127.17, 128.42, 128.88, 129.44, 131.09, 134.28, 137.67, 144.28 (C=N), 157.57 (O-C): MSI MS m/z 311 (M+H, 100 %), 312 (M+1, 32 %). Anal. calcd. for C₁₈H₁₅CIN₂O₂: C, 69.56%; H, 4.86%; N, 9.01%. Found: C, 69.61%; H, 5.08%; N, 9.13%.

1-(4-fluorophenyl)-2-((6-methoxynaphthalen-2-yl)methylene)hydrazine (1g)

Yield 85.03%, m.p. 206 °C; 1H-NMR: δ 3.85 (s, 3H, O-CH₃); 7.03-7.92 (m, 10H Ar-H); 7.96 (s, 1H, HC=N); 10.32 (s, 1H, NH); 13C-NMR: δ 55.20 (OCH₃); 106.31, 112.85, 115.48, 115.70, 118.83, 123.01, 125.72, 127.15, 128.46, 129.39, 131.31, 134.17, 136.88, 142.08 (C=N), 154.65, 156.97, 157.49 (O-C): MSI MS m/z 295 (M+H, 100 %), 296 (M+1, 28 %). Anal. calcd. for C₁₈H₁₅FNO₂: C, 73.45%; H, 5.14%; N, 9.52%. Found: C, 73.63%; H, 5.32%; N, 9.61%.

1-(2,4-dimethylphenyl)-2-((6-methoxynaphthalen-2-yl)methylene)hydrazine (1h)

Yield 82.26%, m.p. 169 °C; 1H-NMR: δ 2.18 (s, 6H, Ar-CH₃), 3.86 (s, 3H, OCH₃), 6.86-7.92 (m, 9H, Ar-H); 7.92 (s, 1H, NH); 13C-NMR: δ 17.37 (Ar-CH₃), 20.11; (Ar-CH₃), 55.17 (OCH₃), 106.30, 112.23, 118.74, 120.60, 123.04, 125.49, 127.06, 127.12, 128.47, 129.36, 130.88, 131.54, 134.11, 136.77, 141.00 (C=N), 157.41 (O-C); MSI MS m/z 305 (M+H, 100 %), 306 (M+1, 32 %). Anal. calcd. for C₂₀H₂₁N₂O: C, 78.92%; H, 6.62%; N, 9.20%. Found: C, 78.39%; H, 6.76%; N, 8.77%.

1-(3,4-dimethylphenyl)-2-((6-methoxynaphthalen-2-yl)methylene)hydrazine (1i)

Yield 83.87%, m.p. 230 °C; 1H-NMR: δ 2.11 (s, 3H, Ar-CH₃), 2.18 (s, 3H, Ar-CH₃), 3.85 (s, 3H, OCH₃), 6.80-7.90 (m, 9H, Ar-H), 7.92 (s, 1H, NH); 13C-NMR: δ 18.55 (Ar-CH₃), 19.76 (Ar-CH₃), 55.19 (OCH₃), 106.31, 109.48, 113.30, 118.78, 123.02, 125.41, 125.99, 127.12, 128.50, 129.34, 131.03, 131.56, 134.04, 135.95, 136.62, 143.39 (C=N), 157.39 (O-C); MSI MS m/z 305 (M+H, 100 %), 306 (M+1, 65 %). Anal. calcd. for C₂₀H₂₁N₂O: C, 78.92%; H, 6.62%; N, 9.20%. Found: C, 78.42%; H, 6.46%; N, 9.18%.

1-(6-methoxynaphthalen-2-ylmethylene)-2-phenyldihydrazine (1j)

Yield 90.68%, m.p. 229 °C; 1H-NMR: δ 3.86 (s, 3H, OCH₃); 6.72-7.93 (m, 11H Ar-H); 7.97 (s, 1H, HC=N); 10.29 (s, 1H, NH); 13C-NMR: δ 55.20 (OCH₃); 106.31, 111.94, 118.60, 118.82, 123.03, 125.69, 127.15, 128.46, 129.10, 129.40, 131.38, 134.16, 136.79, 145.40 (C=N), 157.47 (O-C); MSI MS m/z 277 (M+H, 100 %). Anal. calcd. for C₁₈H₁₆N₂O: C, 78.24%; H, 5.84%; N, 10.14%. Found: C, 77.99%; H, 5.84%; N, 10.10%.
Bioisosteric modification on melatonin

C, 62.79%; H, 4.03%; N, 8.61%. Found: C, 62.03%; H, 4.03%; N, 8.61%. Yield 83.10%, m.p. 163 °C; ¹H-NMR: δ 6.72-8.02 (m, 11H ArH); 8.46 (s, 1H, HC=N); 9.77 (s, 1H, NH); ¹³C-NMR: δ 106.10, 114.63, 120.40, 122.54, 126.35, 126.56, 126.60, 127.70, 128.00, 128.30, 128.60, 132.59, 133.07, 133.09, 133.19, 140.52, 142.39 (C=N): MSI MS m/z 325 (M+, 100 %), 327 (M+2, 100 %). Anal. calced. for C₁₇H₁₃BrN₂: C, 62.79%; H, 4.03%; N, 8.61%. Found: C, 62.61%; H, 4.13%; N, 8.79%.

C₁₇H₁₃ClN₂: C, 72.73%; H, 4.67%; N, 9.98%. Found: C, 72.03%; H, 4.73%; N, 9.88%.

1-(2-bromophenyl)-2-(naphthalen-2-ylmethylene) hydrazine (1k)

Yield 85%, m.p. 223 °C; ¹H-NMR: δ 7.07-7.97 (m, 11H ArH); 8.01 (s, 1H, HC=N); 10.57 (s, 1H, NH); ¹³C-NMR: δ 113.46, 122.04, 122.47, 126.07, 126.16, 126.53, 127.69, 128.86, 128.21, 128.92, 132.88, 133.14, 133.33, 137.35, 144.15 (C=N): MSI MS m/z 281 (M+H, 100 %), 283 (M+2, 32 %). Anal. calced. for C₁₇H₁₃ClN₂: C, 72.73%; H, 4.67%; N, 9.98%. Found: C, 72.03%; H, 4.73%; N, 9.88%.

1-(3-bromophenyl)-2-(naphthalen-2-ylmethylene) hydrazine (1l)

Yield 75.40%, m.p. 175 °C; ¹H-NMR: δ 6.88-7.98 (m, 11H ArH); 8.03 (s, 1H, HC=N); 10.62 (s, 1H, NH); ¹³C-NMR: δ 111.13, 114.12, 121.10, 122.47, 122.49, 126.25, 126.35, 126.53, 127.69, 127.91, 128.26, 131.06, 132.95, 133.10, 133.14, 138.09, 146.78 (C=N): MSI MS m/z 325 (M+, 95 %), 327 (M+2, 100 %). Anal. calced. for C₁₇H₁₃BrN₂: C, 62.79%; H, 4.03%; N, 8.61%. Found: C, 62.64%; H, 4.09%; N, 8.75%.

Yield 77%, m.p. 200 °C; ¹H-NMR: δ 6.50-7.99 (m, 11H Ar-H), 8.03 (s, 1H, HC=N), 10.65 (s, 1H, NH); ¹³C-NMR: δ 98.54, 104.78, 104.99, 108.19, 122.54, 126.24, 145.53, 127.69, 127.90, 128.24, 130.70, 132.94, 133.16, 137.76, 147.23 (C=N), 162.19, 164.58; MSI MS m/z 265 (M+H, 100 %), 266 (M+1, 24 %). Anal. calced. for C₁₇H₁₃F₃N₂: C, 77.25%; H, 4.96%; N, 10.60%. Found: C, 76.91%; H, 5.04%; N, 10.62%.

1-(2-chlorophenyl)-2-(naphthalen-2-ylmethylene) hydrazine (1m)

Yield 85.71%, m.p. 168 °C; ¹H-NMR: δ 6.77-8.01 (m, 11H ArH); 8.43 (s, 1H, HC=N); 10.01 (s, 1H, NH); ¹³C-NMR: δ 114.13, 116.20, 119.69, 122.51, 126.34, 126.55, 126.57, 127.68, 127.98, 128.05, 128.30, 129.36, 133.06, 133.10, 133.20, 140.33, 141.35 (C=N): MSI MS m/z 281 (M+H, 100 %), 283 (M+2, 34 %). Anal. calced. for C₁₇H₁₃ClN₂: C, 72.73%; H, 4.67%; N, 9.98%. Found: C, 72.65%; H, 4.76%; N, 10.02%.

1-(3-chlorophenyl)-2-(naphthalen-2-ylmethylene) hydrazine (1o)

Yield 82.14%, m.p. 172 °C; ¹H-NMR: δ 6.75-7.99 (m, 11H ArH); 8.03 (s, 1H, HC=N); 10.63 (s, 1H, NH); ¹³C-NMR: δ 111.24, 111.72, 118.68, 122.98, 126.73, 126.81, 127.02, 128.17, 128.39, 128.74, 131.24, 133.43, 133.58, 133.62, 134.35, 138.51, 147.14 (C=N): MSI MS m/z 281 (M+H, 100 %), 283 (M+2, 32 %). Anal. calced. for C₁₇H₁₃ClN₂: C, 72.73%; H, 4.67%; N, 9.98%. Found: C, 72.03%; H, 4.73%; N, 9.88%.

1-(4-chlorophenyl)-2-(naphthalen-2-ylmethylene) hydrazine (1p)

Yield 90.90%, m.p. 232 °C; ¹H-NMR: δ 7.04-7.98 (m, 11H Ar-H), 8.00 (s, 1H, HC=N), 10.43 (s, 1H, NH); ¹³C-NMR: δ 112.99, 115.75, 122.47, 125.77, 126.50, 127.67, 127.81, 128.17, 132.79, 133.17, 136.53, 141.91 (C=N), 154.77, 157.09; MSI MS m/z 265 (M+H, 100 %), 266 (M+1, 30 %). Anal. calced. for C₁₇H₁₃F₃N₂: C, 77.25%; H, 4.96%; N, 10.60%. Found: C, 76.01%; H, 5.01%; N, 10.79%.

1-(2,4-dimethylphenyl)-2-(naphthalen-2-ylmethylene) hydrazine (1s)

Yield 85.71%, m.p. 178 °C; ¹H-NMR: δ 2.18 (s, 3H, Ar-CH₃), 2.20 (s, 3H, Ar-CH₃), 6.87-7.98 (m, 10H, ArH), 8.24 (s, 1H, HC=N), 9.57 (s, 1H, NH); ¹³C-NMR: δ 17.43 (Ar-CH₃), 20.17 (Ar-CH₃), 112.37, 120.76, 122.52, 125.58, 125.98, 126.47, 127.14, 127.40, 127.66, 127.82, 128.19, 130.96, 132.77, 133.23, 133.81, 137.33, 140.88 (C=N): MSI MS m/z 275 (M+H, 100 %). Anal. calced. for C₁₇H₁₃N₂: C, 83.18%; H, 6.61%; N, 10.21%. Found: C, 82.58%; H, 6.64%; N, 10.14%.
1-(3,4-dimethylphenyl)-2-(naphthalen-2-ylmethylene) hydrazine (1t).
Yield 78.57%, m.p. 218 °C; 1H-NMR: δ 2.12 (s, 3H, Ar-CH₃), 2.18 (s, 3H, Ar-CH₃), 6.82-7.98 (m, 10H, Ar-H), 7.85 (s, 1H, HCN=), 10.25 (s, 1H, NH); 13C-NMR: δ 18.55 (Ar-CH₃), 19.76 (Ar-CH₃), 109.57, 113.38, 122.48, 125.42, 125.88, 126.22, 126.43, 127.65, 127.75, 128.11, 130.04, 132.66, 133.22, 133.78, 135.58, 136.65, 143.22 (C=NI); MSI MS m/z 275 (M+H, 100 %). Anal. calcd. for C₁₉H₁₈N₂: C, 83.18%; H, 6.61%; N, 10.21%. Found: C, 82.90%; H, 6.63%; N, 10.18%.

1-(naphthalen-2-ylmethylene)-2-phenylhydrazine (1u)
Yield 79.67%, m.p. 223 °C; 1H-NMR: δ 6.73-7.98 (m, 12H ArH); 8.01 (s, 1H, HCN=); 10.44 (s, 1H, NH); 13C-NMR: δ 112.04, 118.82, 122.48, 125.74, 126.02, 126.48, 126.67, 127.81, 128.17, 129.12, 132.77, 133.18, 133.61, 136.44, 145.21 (C=NI); MSI MS m/z 247 (M+H, 100 %). Anal. calcd. for C₁₇H₁₄N₂: C, 82.30%; H, 5.79%; N, 11.29%. Found: C, 82.90%; H, 5.733%; N, 11.37%. All the experimental and characterization data of 1u are in agreement with the literature. (Weil, Ostermeier, 1921)

EXPERIMENTAL – BIOLOGICAL ACTIVITY

Cell culture and chemicals
The CHO-K1 cell line (ATCC) was maintained with Dulbecco’s Modified Eagle Serum/F12 Ham (DMEM: F12) which was supplemented with 10% fetal bovine serum (FBS) and 1mM sodium pyruvate at 37 °C and in a humidified atmosphere containing 5% CO₂. Cell culture media and assay chemicals were obtained from Sigma-Aldrich (St. Louis, MO) except for FBS (Gibco, Grand Island, NY) and sodium pyruvate (Santa Cruz Biotech. Inc., TX).

Free radical scavenging activity evaluation with DPPH assay
2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical with a maximum absorbance of 520 nm. DPPH is reduced with the absence of an antioxidant molecule and loses its reactivity. Thereby, the solution with a dark blue color becomes colorless (Blois, 1958). The DPPH assay was performed just as our previous protocol (Carocci et al., 2014). The novel compounds were added into 150 µL of DPPH methanol: water (4:1) solution with final concentrations of 10µM and 100µM in 96-well plates. DPPH reduction was monitored in a microplate reader at a wavelength of 517 nm for 60 minutes. The radical scavenging activities of the molecules (RSA) were calculated as:

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RSA\% = \frac{(A0-A1)}{A0}\times100
\]

A0: absorbance value of solvent control - DPPH solution containing 10% DMSO - A1: molecular absorbance value - 180 µL DPPH solution containing 20 µL molecule of DMSO solution.

Cytotoxicity evaluation with MTT assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a water-soluble tetrazolium salt. MTT is reduced to purple formazan crystals by the mitochondrial succinate dehydrogenase enzyme. Formazan crystals are dissolved in the appropriate solvent, and the optical density is measured in a spectrophotometer (Niles, Moravec, Riss, 2008).

CHO-K1 cells were plated at a concentration of 5000 cells / well in 96-well plates. The cells were incubated for 24 hours in a humidified environment containing 5% CO₂ at 37 °C. The final concentrations of the test substances were added to the cell medium at 10 µM, and incubation was carried out with the test substances for 24 hours. Control (medium), vehicle control (0.1% DMSO) and positive control (171 µM Triton X-100) groups were used in all experiments. After exposure to the test substances, the media were removed, and the wells were washed with phosphate-buffered saline (PBS). The wells which contained the cells were incubated for 4 hours in a MTT solution with a final concentration of 1 mg/mL in the medium (Mossman, 1983). After incubation, the MTT solution was removed from the wells, and the formazan crystals were dissolved in 150 µL of DMSO. Absorbance values were recorded in a Thermo / Varioskan Flash microplate reader at a wavelength of 550 nm. The effect of the novel compounds on cell viability was calculated as % viability in comparison to the vehicle control (the viability of the vehicle control was accepted as 100%).

Statistical analyses
The statistical analyses of the DPPH and MTT experiments were carried out by using the parametric method of “student’s t-test”.

H. Shirinzadeh, M. Ghalia, A. Tascioglu, F. I. Adjali, G. Gunesacar, H. Gurer-Orhan, S. Suzen
RESULTS

Antioxidant activities of novel compounds

The radical scavenging activities of the novel compounds were investigated by the DPPH assay at final concentrations of 10 μM and 100 μM (Figure 1). According to these results, all molecules exhibited statistically significant radical scavenging activity in comparison to the vehicle control.

Effects on cell viability of novel compounds

The effects of the molecules on cell viability were demonstrated by the MTT assay. The antiproliferative effects of the molecules were investigated at the final concentration of 10 μM in 0.1% DMSO (Figure 3).

DISCUSSION AND CONCLUSION

Biological processes such as aging are thought to play a role in oxidative stress in the pathology of many diseases such as diabetes, atherosclerosis, rheumatoid arthritis and cancer. Therefore, external antioxidant supplementation and/or strengthening of the body’s antioxidant defense system are proposed as treatment approaches for these diseases. One of the mechanisms of action of antioxidant molecules is cleaning/scavenging of reactive oxygen derivatives. In this study, novel compounds were synthesized by bioisosteric modification of the indole ring of MLT, and the possible antioxidant potentials of these compounds were studied by investigating their radical scavenging activities. The radical scavenging activities of the 1-(halogenated phenyl)-2-((6-methoxynaphthalen-2-yl)methylene)hydrazine derivatives (1a-j) were higher than those of the 1-(halogenated phenyl)-2-naphthalen-2-yl)methylene)hydrazine (1k-u) derivatives. It is believed that the 6-methoxy substitution process in these halogen-bearing derivatives may have increased their activity. In the halogen-free derivatives, methoxy substitution (1i, 1j) was not found to cause an increase in activity in comparison to the non-methoxy-substituted derivatives (1t, 1u). The presence of halogen in the o-position reduced the radical scavenging activity (1a, 1d, 1l & 1o), but m- and p-halogen substitution increased the activity of the phenyl substitution in the hydrazine group.

One of the factors causing the greatest time, cost and labor loss in drug discovery and development studies is...
that a candidate compound that is found at the late stages of studies might be thrown away as all work is finally done, so it is a possibility that the toxic effects of such a candidate compound are not studied. For this reason, the pharmaceutical industry and regulatory agencies recommend in vitro rapid screening tests to be conducted at the early stages of drug discovery and development studies, and accordingly, investigation of the cytotoxicity of such substances will prevent these losses. In the light of these information and proposals, the possible cytotoxic effects of the novel compounds were investigated in a healthy cell line by the MTT assay in our study. The MTT assay detects the viable cells with active metabolism which convert MTT into a purple colored formazan product. Thus, color formation serves as a convenient marker of only the viable cells. The findings indicated that there was no significant structure-activity relationship in the potentially cytotoxic effects of the substances, but the 6-methoxy naphthalene compounds seemed to be more cytotoxic than the non-methoxy naphthalene compounds. Moreover, the ortho halogenation on the phenyl ring was found to decrease the cytotoxic potential of the compounds in general. Some substances that are found to have radical scavenging effects appear to have cytotoxic potency. For this reason, it is recommended to evaluate and confirm the radical scavenging/antioxidant effects in a cell-based in vitro model and determine the cytotoxicity potential of the substances at their effective concentrations. It is also possible to investigate the mechanisms of their cytotoxic effects by using a battery of cytotoxicity assays based on various mechanisms instead of a sole assay, MTT.

**Z,E-isomerism evaluation of the synthesized compounds**

Molecules that have the hydrazono group with other functional groups have some special physical and chemical properties. In most cases, these compounds can exist as either anti-E or syn-Z isomers. The ¹H, ¹³C and ¹⁵N NMR spectroscopic techniques and an X-ray diffraction of single crystals are much more informative tools for the study of the fine structure of hydrazono-compounds including information on Z,E-isomerism and hydrogen bonding.

Studies in the literature with compounds that are structurally similar to naphthalene-hydrazone derivatives showed that these molecules exist as anti-E isomers (Figure 4). The structure of 2-thiophenecarbaldehyde 2-quinolylhydrazone (A) was investigated by Mague et al. (1997) using X-ray Crystallographic methods, and the molecule was found in the anti-E isomer form. Based on our previous study (Yilmaz, Coban, Suzen, 2011), we observed that the twenty-one synthesized indole hydrazide/hydrazone (B) derivatives (except for one derivative) existed as anti-E isomers. Our research on quinolone hydrazine derivatives showed similar results (Puskullu et al., 2015). A series of quinoline-2-carboxaldehydes hydrazones was synthesized (C) and
characterized. None of the compounds showed syn-Z isomers according to the findings of the 1H, 13C and 15N NMR spectroscopic analyses. In this study, we observed similar results with naphthalene derivatives (D). No signals were detected to represent isomerism by the 1H, 13C NMR analyses. These literature data and reports (Soltani Rad, Khalafi-Nezhad, Behrouz, 2010), prove that all of the synthesized compounds are anti-E isomer.

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