Full Paper

Synthesis and Antioxidant Activity of New Tetrahydro-Naphthalene-Indole Derivatives as Retinoid and Melatonin Analogs

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A number of retinoid-related compounds represent classes of antioxidative and proapoptotic agents with promising potential in the treatment of neoplastic diseases. Indeed, the synthetic retinoid amide fenretinide [*N*-(4-hydroxyphenyl)retinamide] induces apoptosis of cancer cells and acts as a chemotherapeutic drug in cancer therapy. In the present work, and as a continuation of our studies on retinoid-type compounds, the synthesis of melatonin retinamide derivatives was studied as a novel series of melatonin retinoids, using the condensation reaction sequence involving tetrahydrotetramethylnaphthalene carboxylic acid and appropriate melatonin-type moieties. Despite of the weak DPPH inhibition activity pattern of the synthesized compounds, some of them showed a strong inhibition on lipid peroxidation (**IVa – b, Va,** and **VIIa – c,** 88, 96, 90, 94, 93, and 86%, respectively at 10^{-4} M concentration) when melatonin (85% at 10^{-4} M concentration) was used as a reference compound.

Keywords: Synthesis / Indoles / Antioxidant activity

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Introduction

Vitamin A (retinol) plays an important role in the control of differentiation and proliferation of various epithelia of the body [1]. Absence of vitamin A can lead to uncontrolled proliferation of epithelial stem cells that fail to differentiate into the normal phenotype in many lining epithelia. Retinol and its metabolites are essential for growth and cell differentiation, particularly of epithelial tissues [2]. Retinoic acid is the physiologically most active metabolite of vitamin A that modulates biological processes involved in embryogenesis, skeletal development, cellular differentiation, and growth – in addition, it can modulate programmed cell death. Retinoids play essential roles in many diverse biological events including cell differentiation/proliferation [3] and lipid peroxidase inhibition [4]. It has been shown that Am580 (Fig. 1), a stable retinobenzoic derivative, originally synthesized as a retinoic acid receptor (RAR) alpha agonist, is a powerful inducer of granulocytic maturation in NB4, an APL-derived cell line [5]. Am580 is a potent synthetic retinoic acid derivative showing selectivity towards one of the retinoic acid receptors. It is known that several RAR agonists have therapeutic activity against a variety of cancer types. However, unacceptable toxicity profiles have hindered their development as drugs [6]. RAR agonists presenting novel structural and chemical features could therefore open new avenues for the discovery of leads against breast, lung, and prostate cancer or leukemia.

On the other hand, previous studies have shown that fenretinide [*N*-(4-hydroxyphenyl)retinamide], a synthetic retinoid amide, exhibits antioxidant activities that include scavenging DPPH (2,2,diphenyl-1-picrylhydrazyl) radicals, inhibiting linoleic acid peroxidation initiated by hydroxyl radicals and reducing lipid peroxidation in rat liver microsomes to the same extent or greater than

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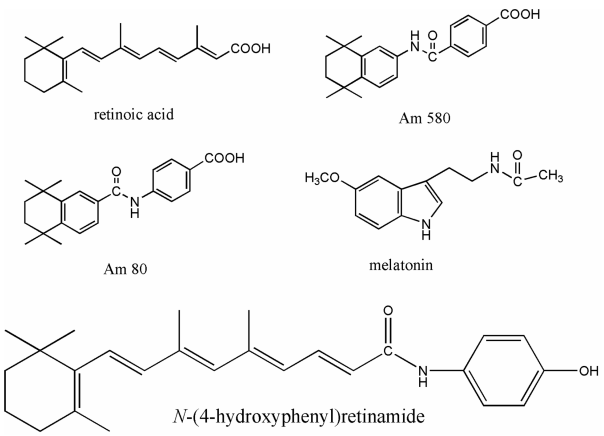
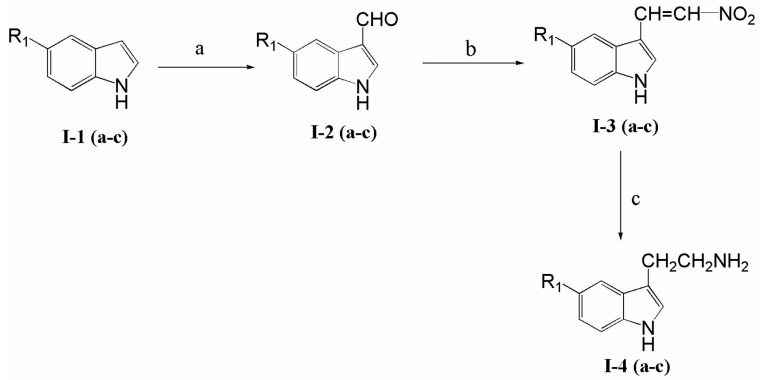


Figure 1. Structures of *all-trans*-retinoic acid, Am 580, Am 80, melatonin, and *N*-(4-hydroxyphenyl)retinamide.

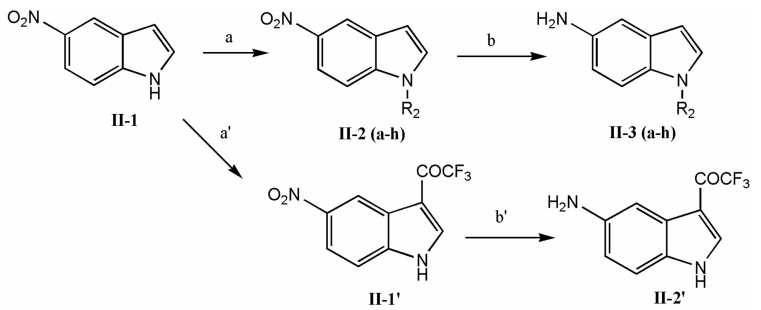
α-tocopherol [7]. Melatonin is also a well-known indole compound having the properties of scavenging free radicals [8], protecting against DNA strand breaks, and lipid peroxidation [9]. Melatonin stimulates several antioxidative enzymes, namely superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GRd), increasing their antioxidant effectiveness [10]. Its potential usefulness in a number of therapeutic areas such as those related to the desynchronization of biological rhythms *e.g.* jet-lag, disturbed sleep-wake cycles [11], seasonal disorders, and depression, is known [12]. Melatonin may also play a role in the cardiovascular system [13]. In this study, we have synthesized novel melatonin retinamide derivatives (containing the melatonin moiety integrated with the structure of retinoic acid and Am580) which can be classified into three sets (Schemes 1–3). The antioxidant activities of these compounds are also reported here.

Results and discussion

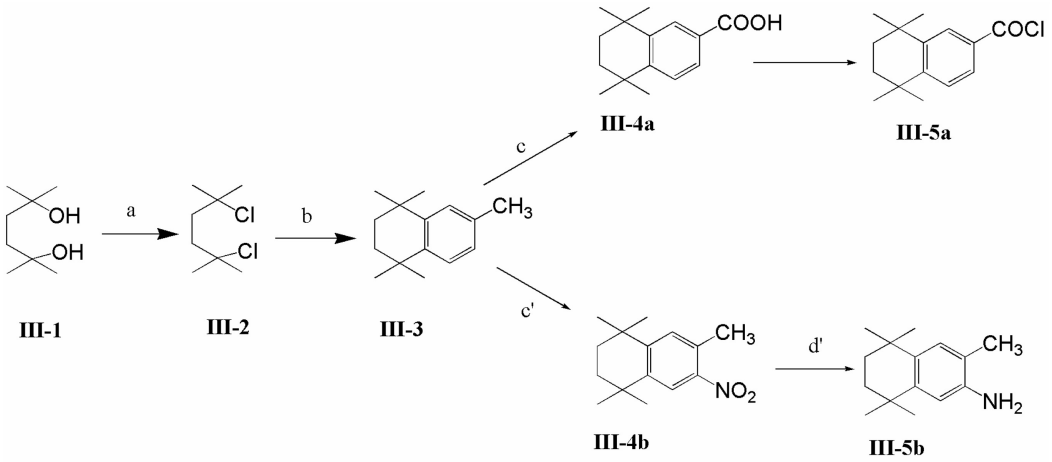
Superoxide anion, DPPH-free radical scavencing activities, and inhibition of lipid peroxidation (LP) assays were used to determine the antioxidative activities of the compounds synthesized. Scavenging effects of synthesized compounds on superoxide anion and DPPH radical and inhibitory effect on the lipid peroxidation are shown in Table 1. The compounds had a strong effect on the lipid peroxidation and did not show a strong effect on the DPPH radical, whereas they showed moderate scavenger effect on superoxide anion when compared with melatonin. The scavenger effects of synthesized compounds on DPPH radical and inhibitory effect on the lipid peroxidation were not similar. Since the mechanisms of these methods are different in manner, it is quite difficult to explain the observed different effects of synthesized compounds unless the fact that further mechanistic studies should be considered. However, the conflicting results obtained from antioxidative capacity and activity may be dependent on several factors: 1) the physical structure of the test systems, 2) the mode of initiating oxidation, 3)



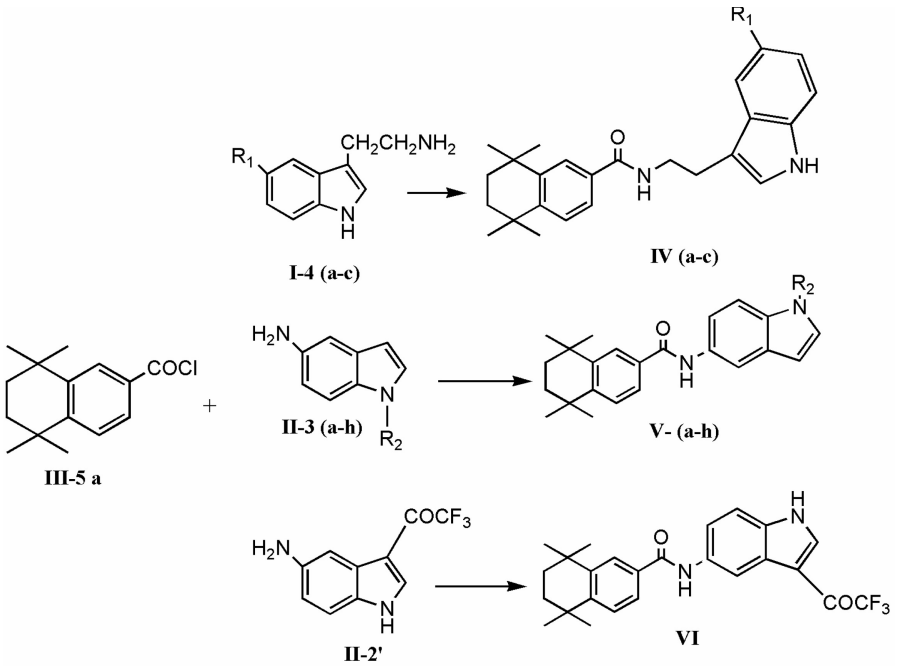
Scheme 1. Synthesis of the compounds **I-4 (a–c)**. R_1 = –H, $-OCH_3$, –Br. a) $POCl_3$, DMF; b) CH_3NO_2, $AcONH_4$; c) $LiAlH_4$



Scheme 2. Synthesis of compounds **II-3** (**a–h**) comprising the 2nd group. R_2 = –H, $-CH_3$, $-C_2H_5$, -*i*-pr, *n*-Bu, -Bn, *p*-fluor-Bn, *o, p*-dichlor-Bn. a) R_2X, DMF, NaH; b) Pd/C (10%), H_2; a′) trifluoroacetic anhydride, DMF, 0°C; b′) Pd/C (10%), H_2

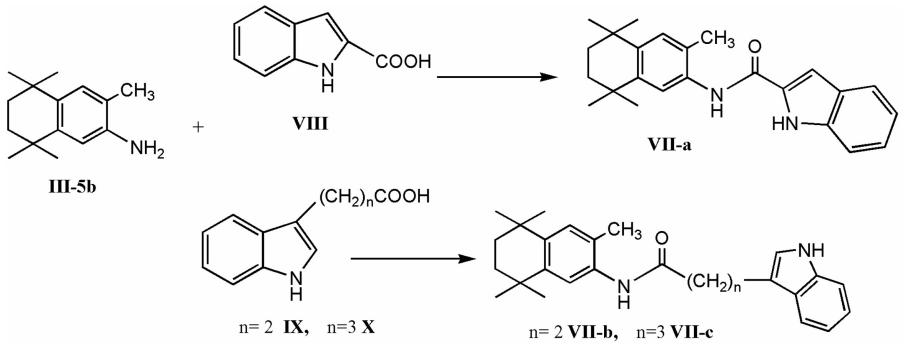


Scheme 3. Synthesis of compounds **III-5** comprising the 3rd group. a) HCl, H_2SO_4, EtOH; b) toluene, $AlCl_3$, $CHCl_2$; c) $KMnO_4$, pyridin, NaOH; c′) HNO_3, H_2SO_4; d′) Pd/C (10%), EtOH



Scheme 4. Amidification reactions of compound (**III-5a**). $SOCl_2$, benzene, $CHCl_2$, triethylamine. R_1 = –H, –OCH_3, –Br; R_2 = –H, –CH_3, –C_2H_5, -*i*-Pr, *n*-Bu, -Bn, *p*-F-Bn, *o, p*-diCl-Bn

the nature of the substrate for oxidation [31]. Compounds **Ve**, **Vg**, **VIIa**, **VIIc**, **Vh**, and **VI** (see Schemes 4 and 5) slightly scavenged the level of the DPPH radical at 10^{-3} M concentration by about 35, 68, 34, 48, and 19%, respectively. Compounds **IVa–c**, **Va–b**, **Vg**, and **VIIa–c** (see Schemes 4 and 5) showed a strong inhibitory effect on the lipid peroxidation at the 10^{-3} M concentration and the inhibition rates were in the range of 90–99%. In addition, these compounds showed even better activity than melatonin at 10^{-3} M concentration. Our results indicate that introduction of a retinoid moiety (1,1,4,4-tetrahydro-1,1,4,4-tetramethylnaphthalene) in place of the methyl portion of the melatonin side chain (Compound **IVb**) produced a compound of high interest in view of its LP inhibitory activity. A difference exists between the substitution pattern on the phenyl ring of the compounds **IVa–c**, in that the non-substituted **IVa** and that substituted with Br, **IVc**, cannot be considered as devoid of LP inhibitory activity, whereas the compound **IVb** retained the best activity within these three compounds at both, 10^{-3} and 10^{-4} M concentrations. In contrast, we found that the compounds **Va–h** in which the amide moiety of the retinoid portion is linked to the indole ring from the fifth position, showed variable results on the



Scheme 5. Amidification reactions of compound (**III-5b**). Reaction conditions: *N,N′*-CDI, DMF

Table 1. Effects of the compounds **IVa–VIIc** on LP levels and scavenging activity of superoxide and DPPH radical*.

Compound Nº	Concentration in incubation medium [M]	Superoxide anion ($O_2^{\cdot-}$) scavenging capacity [% Inhibition]	LP [% Inhibition]	DPPH free radical scavenging activity [% Inhibition]
IVa	10^{-3}	63 ± 2[a)]	93 ± 1	6 ± 2
	10^{-4}	5 ± 5[a)]	88 ± 2	NE[c)]
IVb	10^{-3}	63 ± 3[a)]	98 ± 1	14 ± 3
	10^{-4}	NE[c)]	96 ± 2	2 ± 1
IVc	10^{-3}	47 ± 4[a)]	90 ± 1	2 ± 2[a)]
	10^{-4}	39 ± 2[a)]	26 ± 2[a)]	4 ± 3
Va	10^{-3}	69 ± 2	99 ± 2	10 ± 5
	10^{-4}	59 ± 2[a)]	90 ± 1	3 ± 2
Vb	10^{-3}	71 ± 5	99 ± 1	NE[c)]
	10^{-4}	4 ± 5[a)]	23 ± 4[a)]	NE[c)]
Vc	10^{-3}	64 ± 3	18 ± 5[a)]	5 ± 5
	10^{-4}	4 ± 4[a)]	12 ± 7	1 ± 4
Vd	10^{-3}	73 ± 2	12 ± 8	4 ± 2[a)]
	10^{-4}	15 ± 4[a)]	1 ± 2	1 ± 4
Ve	10^{-3}	71 ± 3	11 ± 3[a)]	35 ± 3
	10^{-4}	NE[c)]	NE[c)]	1 ± 4
Vf	10^{-3}	72 ± 2	7 ± 5[a)]	9 ± 2
	10^{-4}	NE[c)]	NE[c)]	NE[c)]
Vg	10^{-3}	67 ± 4	94 ± 2	68 ± 2[a)]
	10^{-4}	9 ± 6[a)]	5 ± 5[a)]	2 ± 1
Vh	10^{-3}	34 ± 4[a)]	14 ± 1[b)]	19 ± 2
	10^{-4}	28 ± 5	NE[c)]	NE[c)]
VI	10^{-3}	58 ± 2[a)]	31 ± 3[a)]	19 ± 1
	10^{-4}	15 ± 8	16 ± 4[a)]	NE[c)]
VIIa	10^{-3}	52 ± 4[a)]	96 ± 2	34 ± 3
	10^{-4}	12 ± 1[b)]	94 ± 3	NE[c)]
VIIb	10^{-3}	59 ± 12[a)]	98 ± 1	14 ± 2
	10^{-4}	10 ± 6[a)]	93 ± 2	NE[c)]
VIIc	10^{-3}	40 ± 10	96 ± 2	48 ± 4
	10^{-4}	5 ± 12	86 ± 2	NE[c)]
Melatonin	10^{-3}	87 ± 3	87 ± 2	30 ± 4
	10^{-4}	86 ± 4	85 ± 2	5 ± 2

* cf. Experimental section.

a) Significantly different from that of melatonin ($p < 0.05$).

b) Significantly different from that of melatonin ($p < 0.001$).

c) NE = No effect.

lipid peroxidation. Compounds **Vc–e h** and **VI** had slightly inhibited lipid peroxidation by about 18%, 12%, 11%, 14%, and 31%, respectively, at 10^{-3} M concentration, but compound **Vf** had no effect on lipid peroxidation at the same concentration.

Only compound **Va** possessed a notable inhibitory activity when compared to melatonin at 10^{-3} and 10^{-4} M concentrations which are 99% and 90%, respectively. This might indicate the importance of the alkyl chain inclusion at the 1st position on the indole ring. When the benzyl substitution at the 1st position of the indole ring, only the *p*-fluoro compound among them (**Vg**) showing a significant result (94% LP inhibition at 10^{-3} M). The alkyl substitution of the indole ring obviously decresaed the LP inhibition. When compared, for compounds IV and V, different than the alkyl subsitution on the indole ring, it seemed that extending the chain between retinoid amide moiety and the indole ring did not have any influence on the activity in which the compounds from both series had similar LP inhibitory activity, except for those having alkyl substitution on the indole ring.

On the other hand, the results obtained from the compounds **VIIa–c** also showed good LP inhibitory activity in that compound **VIIa** shows extremely high LP inhibition (96% and 94% inh., at 10^{-3} and 10^{-4} M, respectively) in comparison with compounds **VIIb–c**. From these data we can conclude that structural requirements for optimal LP inhibitory activity are rather strict, in particular that (a) the presence of the linker group (amide) between the retinoid head and indole moiety might be required for activity and (b) alkyl residues linked to the indole moiety may be more critical for antioxidant activity than with linker chain. The magnitude of the alkyl substitution seemed also to be a critical factor as the increased volume of substitution led to decrease in activity, with the exception that benzyl substitution showed rather better activity at 10^{-3} M among other bulky substituents examined. However, further investigations are necessary to clarify this issue.

The superoxide anion radical scavenging activities of the synthesized compounds at different concentration were also investigated. The results showed that all synthesized compounds expressed superoxide anion scavenging activity to a certain extent, and the scavenging rates were in the range of 34–73%. Compounds **IVa–b** and **Va–g** have some scavenger effect on superoxide anion at 10^{-3} M concentration (63–73%). Addition-

ally, these compounds had comparable scavenger effects on superoxide radical as that of melatonin (87% and 86% inhibitor at 10^{-3} and 10^{-4} M concentrations, respectively). Compounds **IVc** and **Va** have also decreased the level of superoxide anion by about 39% and 59% at 10^{-4} M concentration. Compound **Va** has optimal scavenging activity for a superoxide radical as it showed 63% and 59% scavenging activity at 10^{-3} and 10^{-4} M concentrations, respectively.

Our studies have revealed information about synthesized melatonin retinamide inhibition of oxygen free radicals in which such retinamides might be of importance in the context of the prevention of cancer diseases, in which the reactive oxygen species may directly or indirectly trigger the expression of redox-sensitive genes in the organism and, consequently, changes in the cellular redox system, have impact on the regulation of gene expression. Despite the promising results of the studys, it is difficult at the moment to directly relate available experimental data to human pathophysiology in terms of chemotherapy of cancer. Nevertheless, and in consideration of the great impact of retinoid antioxidants on cancer disease prevention, further studies in this field are required.

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Experimental

General

Melting points were determined with a Büchi SMP-20 and Buchi 9100 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The ^{1}H-NMR spectra were recorded with a Varian Mercury-400 spectrophotometer (Varian Inc., Palo Alto, CA, USA), in DMSO-d_6 or $CDCl_3$ unless otherwise stated, using the δ scale (ppm) from internal standard TMS. The IR spectra were recorded on a Jasco FT/IR-420 spectrophotometer (JASCO Research Ltd., Victoria, BC, Canada) as potassium bromide pellets. The mass spectra were recorded with a Waters ZQ micromass LC-MS spectrometer (Waters Corporation, Milford, MA, USA) by the method of ES^+. Elemental analyses were performed on LECO 932 CHNS instrument (Leco, St. Joseph, MI, USA) and were within ±0.5% of the theoretical values. Analyses were performed at Scientific and Technical Research Council of Turkey, Instrumental Analysis Center, Ankara, Turkey. Analytical thin-layer chromatography (TLC) was run on Merck silica gel plates (Kieselgel 60F-254). Column chromatographies were accomplished on silica gel 60 (40 – 63 μm particle size) (Merck, Darmstadt, Germany). All starting materials and reagents were high-grade commercial products purchased from Aldrich (Steinheim, Germany), Merck or Fluka (Buchs, Switzerland). Physical and spectral data for compounds **IVa – VIIc** are summarized in Table 2.

Chemistry

Conjugation of the two moieties was through an amide bond which is located between the carboxyl group of 1,1,4,4-tetramethyl-6-carboxy-1,2,3,4-tetrahydro-naphthalene and the amine group on position 3 or 5 of the indole ring.

Syntheses of compounds I-4 (a – c) comprising the 1st group

The syntheses of the compounds **I-4 (a – c)** was carried out as shown in Scheme 1. The indole-3-aldehyde was obtained by direct formylation of indole with dimethylformamide, using phosphorus oxychloride as a catalyst [14 – 15]. Indole-3-carboxaldehydes were refluxed in nitromethane and ammonium acetate to produce 3-(2-nitroethenyl)-1*H*-indoles [16]. This was followed by synthesizing 3-indole-ethylamines using $LiAlH_4$ as described by Faust et al. [17 – 18].

Syntheses of compounds II-3 (a – h) comprising the 2nd group

Syntheses of 1-substituted 5-nitro-1*H*-indole derivatives **II-3 (a – h)** were obtained according to the procedure described by Mor *et al*. [18 – 19], starting from **II-1** and using an alkylating agent. The trifluoroacetic anhydride was added to a solution of 5-nitro-1*H*-indole in dry dimethylformamide at 0°C. The solution was allowed to warm to room temperature and then heated at reflux for 24 h. The reaction mixture was cooled and poured into water. The resulting mixture was filtered and dried. Catalytic hydrogenation of the aromatic nitro group in ethanol was conducted at room temperature and under a pressure of 35 psi using Pd/C (10%) as catalyst. The reaction was filtered through celite to remove the Pd/C, and the solvent was evaporated [20]. **II-1′** and **II-2′** are original compounds. M+1 peaks in mass spectra of these compounds are 259, 229, melting points are 243, 198°C, and yields for these compounds are 62 and 68%, respectively.

Syntheses of compounds III-5 comprising the 3rd group

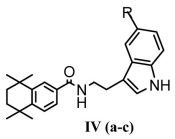
The starting material was the 2,5-dichloro-2,5-dimethylhexane, which was prepared in 56% yield by passing dry hydrochloride gas over 2,5-dimethyl-2,5-hexandiole as described by Boehm et al. [21]. Toluene was alkylated by 2,5-dichloro-2,5-dimethyl hexane in dichloromethane catalyzed with aluminum chloride to produce 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene, in 91% yield [21 – 23]. 1,1,4,4-tetramethyl-6-carboxy-1,2,3,4-tetrahydro-naphthalene, obtained by oxidation of 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene with $KMnO_4$ in 80% yield [24].

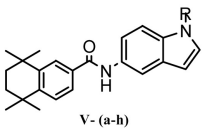
A mixture of HNO_3 and H_2SO_4 was added to 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene (**III-3**) at −10°C to give 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethyl-7-nitronaphthalene in 50% yield. This nitro compound was hydrogenated by the usual method (10% Pd-C in EtOH, 48%) [25].

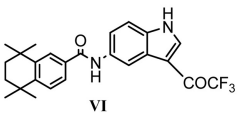
1,1,4,4-Tetramethyl-6-carboxy-1,2,3,4-tetrahydro-naphthalene was acylated with $SOCl_2$ in dry benzene [26]. A crude oil of amines **I-4 (a – c)** and compounds **II-3 (a–h)**, **II-2′** which was then used for amidification with 5,5,8,8-tetramethyl-1,2,3,4-tetrahydronaphthalene-6-carboxylic acid without purification.

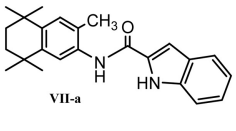
A mixture of appropriate acide and 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethyl-7-aminonaphthalene (**III-5b**) was amidification with *N,N′*-CDI [27]. Compounds **VIII**, **IX**, and **X** are commercially avaliable.

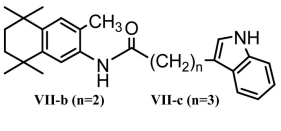
Table 2. Physical and spectral data for compounds **IVa-VIIc**


IV (a-c)


V- (a-h)


VI


VII-a


VII-b (n=2) VII-c (n=3)

N°	R	Mp. [°C]	Yield [%]	Formulas	^{1}H- and ^{13}C-NMR	Mass (ESI+)	IR [cm^{-1}]	Isolation
IVa	H	220	28	$C_{25}H_{30}N_2O \cdot 0.8\ H_2O$	^{1}H-NMR ($CDCl_3$) δ 1.3 (s, 12H), 1.65 (s, 4H), 3.1 (t, 2H), 3.8 (m, 2H), 6.15 (s, 1H), 7.1 – 7.7 (m, 8H), 8.1 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ 166.09, 147.42, 144.18, 136.16, 131.81, 127.21, 126.21, 125.12, 124.25, 122.49, 120.80, 118.21, 118.13, 111.89, 111.27, 34.42, 34.29, 33.97, 33.94, 31.44, 31.32, 25.18	375 (M+1, 100)	Amid I, 1638 Amid II, 1530	$CHCl_3$: *n*-hexane (3 : 1), cc
IVb	OCH_3	190	30	$C_{26}H_{32}N_2O_2$	^{1}H-NMR ($CDCl_3$) δ 1.33 (d, 12H), 1.69 (s, 4H), 3.08 (t, 2H), 3.77 (s, 3H), 3.8 (q, 2H), 6.22 (s, 1H), 6.87 – 8.1 (m, 7H), 8.25 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ 167.98, 154.34, 148.90, 145.62, 132.07, 131.82, 128.02, 126.97, 125.84, 123.77, 123.18, 113.09, 112.79, 112.33, 100.58, 55.98, 40.64, 35.17, 35.05, 34.69, 34.59, 31.93, 25.58	405 (M+1, 100)	Amid I, 1632 Amid II, 1539	$CHCl_3$
IVc	Br	92	42	$C_{25}H_{29}BrN_2O$	^{1}H-NMR ($CDCl_3$) δ 1.28 (s, 12H), 1.68 (s, 4H), 3.04 (t, 2H), 3.77 (m, 2H), 6.16 (s, 1H), 7.16 – 7.8 (m, 7H), 8.24 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ 166.11, 147.43, 144.18, 134.82, 131.75, 129.12, 126.22, 125.10, 124.35, 124.20, 123.24, 120.54, 113.29, 111.81, 110.86, 34.41, 34.29, 33.97, 33.93, 31.44, 31.31, 24.90	455 (M+2, 100), 456 (M+3, 60)	Amid I, 1630 Amid II, 1541	$CHCl_3$: *n*-hexane (7 : 1), cc
Va	H	177	60	$C_{23}H_{26}N_2O \cdot 0.5\ H_2O$	^{1}H-NMR ($CDCl_3$) δ 1.34 (d, 12H), 1.74 (s, 4H), 6.52 (t, 1H), 7.2 – 7.9 (m, 8H), 8.25 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ 165.90, 148.44, 145.12, 133.69, 133.38, 131.63, 128.11, 127.12, 126.55, 126.25, 125.43, 117.08, 113.19, 111.64, 101.80, 35.26, 35.10, 34.88, 34.81, 32.25, 32.16	347 (M+1, 100)	Amid I, 1630 Amid II, 1541	$CHCl_3$
Vb	CH_3	196	32	$C_{24}H_{28}N_2O \cdot 0.3\ H_2O$	^{1}H-NMR ($CDCl_3$) δ 1.3 (d, 12H), 1.75 (s, 4H), 3.8 (s, 3H), 6.45 (d, 1H), 7.1 – 7.95 (m, 8H); ^{13}C-NMR (DMSO-d_6) δ 165.87, 148.45, 145.10, 134.24, 133.28, 131.79, 130.79, 128.41, 127.10, 126.23, 125.39, 116.98, 113.33, 109.93, 100.94, 35.21, 35.07, 34.82, 34.78, 33.21, 32.22, 32.11	361 (M+1, 100)	Amid I, 1647 Amid II, 1531	EtOAc crystallization
Vc	C_2H_5	181	19	$C_{25}H_{30}N_2O \cdot 0.4\ H_2O$	^{1}H-NMR ($CDCl_3$) δ 1.33 (d, 12H), 1.48 (t, 3H) 1.72 (s, 4H), 4.18 (q, 2H) 6.47 (d, 1H), 7.12 – 7.97 (m, 8H); ^{13}C-NMR (DMSO-d_6) δ 165.87, 148.45, 145.10, 133.27, 131.74, 129.13, 128.57, 127.10, 126.23, 125.40, 116.94, 113.46, 109.94, 101.16, 41.02, 35.22, 35.07, 34.82, 34.77, 32.22, 32.12, 16.17	375 (M+1, 100)	Amid I, 1640 Amid II, 1535	*n*-hexane: EtOAc (3 : 1), cc
Vd	*i*-C_3H_7	88	33	$C_{26}H_{32}N_2O \cdot 0.1\ H_2O$	^{1}H-NMR ($CDCl_3$) δ 1.32 (d, 12H), 1.53 (d, 6H) 1.72 (s, 4H), 4.65 (m, 1H), 6.48 (d, 1H), 7.2 – 7.95 (m, 8H); ^{13}C-NMR (DMSO-d_6) δ 165.87, 148.47, 145.12, 133.30, 133.05, 131.79, 128.52, 127.11, 126.26, 125.85, 125.42, 116.84, 113.43, 110.12, 101.46, 47.26, 41.60, 35.23, 35.09, 34.84, 34.79, 32.24, 32.14, 23.21	389 (M+1, 100)	Amid I, 1636 Amid II, 1536	$CHCl_3$: *n*-hexane (2 : 1), cc
Ve	*n*-butyl	149	78	$C_{27}H_{34}N_2O \cdot 0.3\ H_2O$	^{1}H-NMR ($CDCl_3$) δ 0.9 (t, 3H), 1.33 (d, 12H), 1.33 (m, 2H), 1.6 (s, 4H), 1.85 (m, 2H), 4.1 (t, 2H) 6.45 (d, 1H), 7.1 – 7.95 (m, 8H); ^{13}C-NMR (DMSO-d_6) δ 165.86, 148.44, 145.09, 133.55, 133.28, 131.70, 129.80, 128.48, 127.08, 126.23. 125.39, 116.94, 113.45, 110.04, 101.03, 45.95, 35.21, 35.07, 34.81, 34.77, 32.67, 32.22, 32.11, 20.16, 14.22	403 (M+1, 100)	Amid I, 1637 Amid II, 1541	*n*-hexane : EtOAc (5 : 1), cc
Vf	benzyl	105	29	$C_{30}H_{32}N_2O \cdot 0.25\ C_4H_8O_2$	^{1}H-NMR ($CDCl_3$) δ 1.3 (d, 12H), 1.72 (s, 4H), 5.35 (s, 2H), 6.55 (d, 1H), 7.1 – 8.0 (m, 13H); ^{13}C-NMR ($CDCl_3$) δ 166.21, 149.09, 145.86, 137.64, 134.10, 132.88, 129.42, 129.14, 128.98, 127.85, 127.14, 126.90, 126.04, 123.77, 116.44, 113.33, 110.12, 102.12, 50.46, 35.17, 35.05, 34.75, 34.69, 32.02, 31.93	437 (M+1, 100)	Amid I, 1643 Amid II, 1536	*n*-hexane : EtOAc (4 : 1), cc

Table 2. Continued

N°	R	Mp. [°C]	Yield [%]	Formulas	^{1}H- and ^{13}C-NMR	Mass (ESI+)	IR [cm^{-1}]	Isolation
Vg	*p*-fluoro-benzyl	173	63	$C_{30}H_{31}FN_2O \cdot 0.1\ C_2H_5OH \cdot 0.2\ H_2O$	^{1}H-NMR ($CDCl_3$) δ 1.3 (d, 12H), 1.75 (s, 4H), 5.3 (s, 2H), 6.55 (d, 1H), 6.95 – 8.0 (m, 12H); ^{13}C-NMR (DMSO-d_6) δ 165.89, 163.28, 160.87, 148.47, 145.10, 135.22, 133.47, 133.24, 132.05, 130.23, 129.73, 129.65, 128.78, 127.09, 126.23, 125.37, 117.18, 116.06, 115.85, 113.49, 110.43, 101.82, 49.08, 35.20, 35.06, 34.81, 34.76, 32.21, 32.10	455 (M+1, 70), 456 (M+2, 25)	Amid I, 1644 Amid II, 1530	*n*-hexane : EtOAc (3 : 1), cc ethanol crystallization
Vh	*o,p*-dichloro-benzyl	183	24	$C_{30}H_{30}Cl_2N_2O$	^{1}H-NMR ($CDCl_3$) δ 1.33 (d, 12H), 1.75 (s, 4H), 5.36 (s, 2H), 6.6 (d, 1H), 7.1 – 8.05 (m, 11H)	505 (M+1, 94), 507 (M+3, 65), 508 (M+4, 22)	Amid I, 1634 Amid II, 1535	*n*-hexane : EtOAc (5 : 1), cc
VI	–	227	29	$C_{25}H_{25}F_3N_2O_2 \cdot 0.2\ C_4H_8O_2 \cdot 0.4\ H_2O$	^{1}H-NMR (DMSO-d_6) 1.3 (d, 12H), 1.65 (s, 4H), 7.4 – 8.6 (m, 7H), 10.2 (s, 1H), 10.7 (s, 1H)	443 (M+1, 100)	1723 (ketone) Amid I, 1643 Amid II, 1555	*n*-hexane : EtOAc (3 : 1), cc
VIIa	–	135	19	$C_{24}H_{28}N_2O \cdot 0.3\ C_6H_{14} \cdot 0.3\ C_4H_8O_2$	^{1}H-NMR ($CDCl_3$) δ 1.32 (d, 12H), 1.7 (s, 4H), 2.33 (s, 3H), 6.97 (s, 1H), 7.11 – 7.87 (m, 7H), 9.7 (s, 1H)	361 (M+1, 100)	Amid I, 1638 Amid II, 1537	*n*-hexane : EtOAc (3 : 1), cc
VIIb	–	131	22	$C_{26}H_{32}N_2O \cdot 0.2\ H_2O$	^{1}H-NMR ($CDCl_3$) δ 1.25 (d, 12H), 1.65 (s, 4H), 1.92 (s, 3H), 2.8 (t, 2H), 3.25 (t, 2H) 6.75 (s, 1H), 7.1 – 7.7 (m, 7H), 8.1 (s, 1H)	389 (M+1, 100)	Amid I, 1644 Amid II, 1522	*n*-hexane : EtOAc (2 : 1), cc
VIIc	–	158	25	$C_{27}H_{34}N_2O \cdot 0.7\ H_2O$	^{1}H-NMR ($CDCl_3$) δ 1.3 (d, 12H), 1.65 (s, 4H), 2.2 (s, 3H), 2.2 (m, 2H), 2.42 (t, 2H), 2.9 (t, 2H), 6.81 (s, 1H), 7.1–7.75 (m, 7H), 8.05 (s, 1H)	403 (M+1, 100)	Amid I, 1711 Amid II, 1536	*n*-hexane : EtOAc (3 : 1), cc

ESI: Elektrospray ionization, cc: column chromatography, C_6H_{14}: *n*-hexane, $C_4H_8O_2$: EtOAc, C_2H_5OH: ethanol.
Elemental analyses were within ±0.5%.

General method for 5,5,8,8-tetramethyl-5,6,7,8-tetra-hydro-naphthalene-2-carboxylic acid-(substituted-1H-indol)-amide derivatives

A mixture of 1,1,4,4-tetramethyl-6-carboxy-1,2,3,4-tetrahydro-naphthalene (**III-4a**) (1 mmol), thionyl chloride (2 mL), and benzene (6 mL) was heated at reflux for 4 h. After removal of the solvent, a mixture of substituted-indole-amine (1 mmol), chloroform (5 mL), triethylamine (0.5 mL) was added to the residue and the whole mixture was stirred and refluxed overnight. The reaction mixture was evaporated, washed with Na_2CO_3 (5%), extracted with ethyl acetate, and washed with water. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel flash column chromatography [26].

General method for indol-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalene-2yl)-amide derivatives

A mixture of appropriate acid (0.6 mmol) and *N,N′*-CDI (0.65 mmol) in DMF (10 mL) was stirred for 4 h. 1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethyl-7-amine-naphthalene (**III-5b**) (0.6 mmol) was added and stirred for 3 h. The reaction mixture was evaporated, extracted with ethyl acetate, and washed with water. The organic layer was dried over Na_2SO_4, and evaporated. The residue was purified by silica gel flash column chromatography [27].

Antioxidant activity studies

Chemicals used in these experiments include: xanthine, xanthine oxidase, cytochrome *c*, 2,2,diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene, and α-tocopherol thiobarbituric acid (TBA) which were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Superoxide radical scavenging activity

The capacity of melatonin retinoid compounds to scavenge superoxide anion formation was determined spectrophotometrically on the basis of inhibition of cytochrome *c* reduction according to the modified method of McCord *et al*. [28]. Superoxide anion was generated in the xanthine/xanthine oxidase system. The reaction mixture contained in a final volume of 1 mL, 0.05 M phosphate buffer pH 7.8, 0.32 U xanthine oxidase, 50 μM xanthine, 60 mM ctytochrome *c*, and different concentrations of synthesized compounds at 100 μL. The absorbance was measured spectrophotometrically at 550 nm for cytochrome *c* reduction. Each experiment was performed in triplicate, and the results are expressed as a percent of the control.

DPPH radical scavenging assay

The free radical scavenging activities of these compounds were tested by their ability to bleach the stable radical 2,2,diphenyl-1-picrylhydrazyl (DPPH) [29]. Because of its odd electron, DPPH gives a strong absorption band at 517 nm in visible spectroscopy. The reaction mixture contained 100 μM DPPH in methanol and different concentrations of synthesized compounds. Absorbance at 517 nm was determined after 30 min at room temperature and the scavenging activity was calculated as a percentage of the radical reduction. Each experiment was performed in triplicate. Melatonin was used as a reference compound.

Assay of lipid peroxidation

The effect of synthesized compounds on rat liver homogenate induced with $FeCl_2$-ascorbic acid and lipid peroxidation was determined by the method modified by Mihara *et al*. [30]. Wistar rats (200 – 225 g; Refik Saydam Hygiene Center, Ankara, Turkey) were fed with standard laboratory rat chow and tap water *ad libitum*. The animals were starved for 24 h prior to sacrifice and then killed by decapitation under anaesthesia. The livers were removed immediately, washed in ice-cold distilled water, and homogenized immediately with teflon homogenizer in chilled ice. LP was measured spectrophotometrically by estimation of thiobarbituric acid reactant substances (TBARS). Amounts of TBARS were expressed in terms of nmol malondialdehyde

(MDA)/g tissue. A typical optimized assay mixture contained 0.5 mL of liver homogenate, 0.1 mL of Tris-HCl buffer (pH 7.2), 0.05 mL of 0.1 mM ascorbic acid, 0.05 mL of 4 mM $FeCl_2$, and 0.05 mL of various concentrations of synthesized compounds, or *a*-tocopherol incubated for 1 h at 37°C. After incubation, 3.0 mL of H_3PO_4 and 1 mL of 0.6% TBA were added and shaken vigorously. The mixture was boiled for 30 min. After cooling, *n*-butanol was added and the mixture was shaken vigorously. The *n*-butanol phase was separated by centrifugation at 3000 rpm for 10 min. The absorbance of the supernatant was read at 532 nm against a blank, which contained all reagents except liver homogenate.

Statistical analysis

Data were compared by using Student's t-test. All results are presented as means ± SD.

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