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Research Article

Design and Synthesis of Sustain-Acting Melatonin Prodrugs

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Twelve melatonin amide prodrugs aiming at prolonging the action of melatonin *in vivo* by improving its half-life were designed and synthesized. Using an 80% human plasma model, it was found that the aliphatic amide derivatives were relatively stable and melatonin release from these compounds was not sufficient with melatonin release percentage. After 4-hour incubation with 80% human plasma, the melatonin release percentage achieved only approximately less than 20%. In contrast, the N^1-succinyl and N^1-glutaroylmelatonin derivatives (compounds **11** and **12**, resp.) were found to release melatonin in much higher rates. After 3-hour incubation in 80% human plasma, the melatonin release rates from **11** and **12** were found to be 67.3 and 75.6%, respectively. From these results, the N^1-succinyl and N^1-glutaroylmelatonin derivatives (compounds **11** and **12**) could be promising as sustained release prodrugs of melatonin.

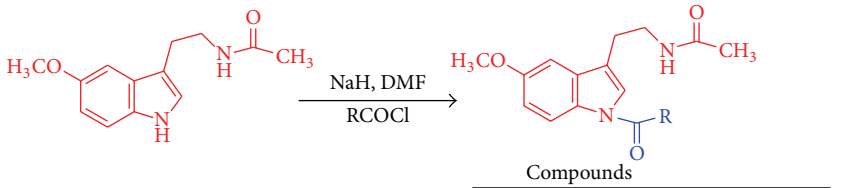
1. Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine, Figure 1) is a hormone produced in the pineal gland located in the center of the brain. It is interesting that the secretion of melatonin is not restricted to mammals, but it is also produced in nonmammalian vertebrates, in some invertebrates, and even in many plants, with the same molecular structure [1]. It is one among many endogenous body environment sensors. Interest in this small molecule has increased dramatically in the past several decades. As a result, a number of important biological actions of melatonin have been reported. Several studies have demonstrated that this compound can act as a maternal fetal synchronizer [2] or a chemical mediator of photoperiodic information [3, 4]. Melatonin is also a modulator of various endocrinological, neurophysiological, and behavioral functions in vertebrates [5–8]. Especially, melatonin has been shown to regulate retinal vertebrate physiology and to function as an inducer of sleep in humans. In this respect, melatonin acts by a unique mechanism of action by targeting specific receptors in the brain that are responsible for controlling the body's sleep-wake cycle [5]. In addition, this compound can influence the circadian rhythm in reptiles, birds, and mammals including human. Recently, it has been observed that melatonin level is found to decline with age. This observation leads many scientists to believe that the lack of melatonin may play a role in the development of age related disorders. Among the diseases that melatonin is thought to affect are Alzheimer's disease, glucose intolerance, impaired immune function, and cancer [9].

Due to diverse biological properties mentioned above, melatonin has received a great deal of attention in the last decade. Various forms of over-the-counter drugs or food supplements are available with therapeutic indications including synchronization of disturbed circadian rhythms such as jet-lag [10], sleep-wake cycle [11], seasonal disorders [12], and winter depression [13].

In clinical setting, it is noted that toxicity of melatonin is remarkably low, and no serious negative side effects of melatonin have been reported, so far [14]. The main disadvantage of melatonin is its poor pharmacokinetic profile. Though the bioavailability of melatonin achieves 30–50%, *in vivo*, melatonin is quickly metabolized with a half-life of only 30 to 50 minutes [15], which greatly limits its clinical value.

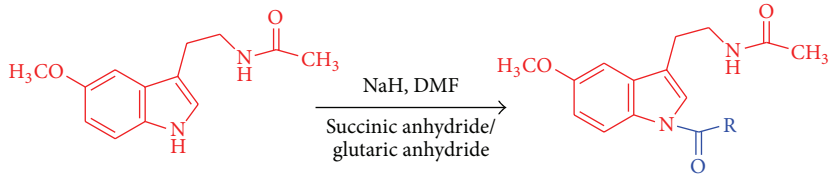
With the aim to improve the pharmacokinetic profile of melatonin, we have designed a series of sustain-acting melatonin prodrugs (Figure 2). This paper reports the results of synthesis and preliminary evaluation of melatonin release from these potential prodrugs.



Compounds

(1) R = $-CH_3$ **(6)** R = n$-C_6H_{13}$
(2) R = $-C_2H_5$ **(7)** R = n$-C_7H_{15}$
(3) R = n$-C_3H_7$ **(8)** R = n$-C_8H_{17}$
(4) R = n$-C_4H_9$ **(9)** R = n$-C_9H_{19}$
(5) R = n$-C_5H_{11}$ **(10)** R = $-OCH_2CH(CH_3)_2$

Scheme 1: Synthesis of N^1-acylmelatonin derivatives.



(11) R = $-CH_2CH_2COOH$
(12) R = $-CH_2CH_2CH_2COOH$

Scheme 2: Synthesis of N^1-succinoyl and N^1-glutaroylmelatonin derivatives.

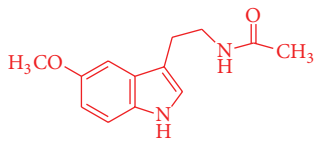
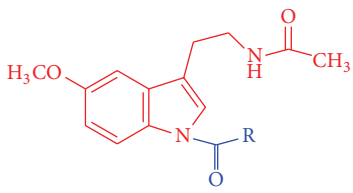


Figure 1: Structure of melatonin.



Compounds

(1) R = $-CH_3$ **(7)** R = n$-C_7H_{15}$
(2) R = $-C_2H_5$ **(8)** R = n$-C_8H_{17}$
(3) R = n$-C_3H_7$ **(9)** R = n$-C_9H_{19}$
(4) R = n$-C_4H_9$ **(10)** R = $-OCH_2CH(CH_3)_2$
(5) R = n$-C_5H_{11}$ **(11)** R = $-CH_2CH_2COOH$
(6) R = n$-C_6H_{13}$ **(12)** R = $-CH_2CH_2CH_2COOH$

Figure 2: Designed melatonin prodrugs.

2. Results and Discussion

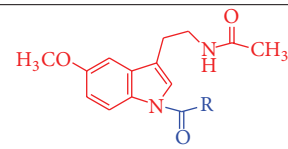
Compounds **1**–**10**, the N^1-acylated melatonin derivatives with acyl chains from 2 to 10 C (**1**–**9**) or s-butyloxycarbonyl (**10**), were synthesized from melatonin by reacting with appropriate acyl chlorides in dimethylformamide used as a solvent (Scheme 1). Conventional methods using pyridine or triethylamine as bases were not successful. It was found that sodium hydride gave the best results, affording the target compounds in average to good yields (65–87%). Among the N^1-acylated melatonin derivatives (**1**–**9**), compound **1** had been synthesized previously [16].

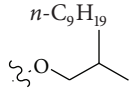
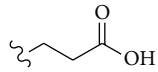
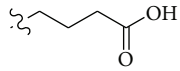
Two additional compounds **11** and **12** bearing succinyl and glutaryl moieties were synthesized by a similar protocol, just using succinic anhydride or glutaric anhydride instead of acyl chloride (Scheme 2).

All compounds were unambiguously identified by analysis of spectral data, including IR, MS, ^{1}H-NMR, and ^{13}C-NMR. In the IR spectra, all characteristic absorption bands of functional groups clearly appeared, for example, ~3325 (NH), ~3010–3220 (CH-aromatic), ~2930, 2910 (CH_3, CH_2), ~1670–1720 (C=O), ~1610, 1580, 1475 (C=C, aromatic), and ~1250 (C–O). ESI mass spectra showed strong molecular peaks and a few fragments appropriately matching the corresponding structures. In the ^{1}H-NMR and ^{13}C-NMR, besides the characteristic peaks of the melatonin skeleton, all protons and carbons of the newly added N^1-acyl moieties were observed.

All the synthesized compounds were preliminarily evaluated for the ability to release melatonin using an 80% human plasma assay [17]. In these experiments, each compound was incubated with an 80% human plasma solution (pH 7.4). After each interval of time (1, 2, 3, and 4 hours) an aliquot of 500 μL from each compound's solution was withdrawn and processed to give a clear supernatant which was then analyzed by HPLC to determine the melatonin release rate. It was found that compounds **1**–**10** did not release melatonin efficiently. After 4 hours, the percentages of melatonin release from these compounds achieved only less than 20% (Table 1). For intended use of these compounds as sleep inducers, these melatonin release percentages were clearly not appropriate.

Table 1: Percentages of melatonin release from compounds **1–12** after incubation with 80% human plasma for different time intervals.



Cpd	R	Percentage (%) of melatonin released after incubation time of			
		1 hour	2 hours	3 hours	4 hours
1	$-CH_3$	—	7.5 ± 1.1	15.4 ± 1.3	18.9 ± 1.7
2	$-CH_2CH_5$	—	7.5 ± 1.2	15.8 ± 1.2	17.8 ± 1.5
3	n-C_3H_7	—	7.1 ± 0.9	14.9 ± 0.9	19.4 ± 2.0
4	n-C_4H_9	—	6.5 ± 0.7	15.2 ± 1.3	17.4 ± 1.9
5	n-C_5H_{11}	—	6.7 ± 0.5	15.0 ± 1.0	18.5 ± 2.1
6	n-C_6H_{13}	—	6.3 ± 0.5	14.7 ± 0.8	17.6 ± 1.7
7	n-C_7H_{15}	—	5.9 ± 0.5	14.2 ± 1.4	16.3 ± 1.0
8	n-C_8H_{17}	—	5.6 ± 0.5	14.4 ± 1.2	16.0 ± 1.1
9	n-C_9H_{19}	—	5.1 ± 0.5	13.9 ± 1.0	15.3 ± 0.9
10		15.7 ± 1.5	26.9 ± 2.1	38.2 ± 2.9	44.7 ± 3.2
11		25.4 ± 2.2	37.8 ± 2.9	64.4 ± 3.2	75.2 ± 3.8
12		31.5 ± 2.4	44.1 ± 3.7	67.3 ± 3.5	83.5 ± 4.2

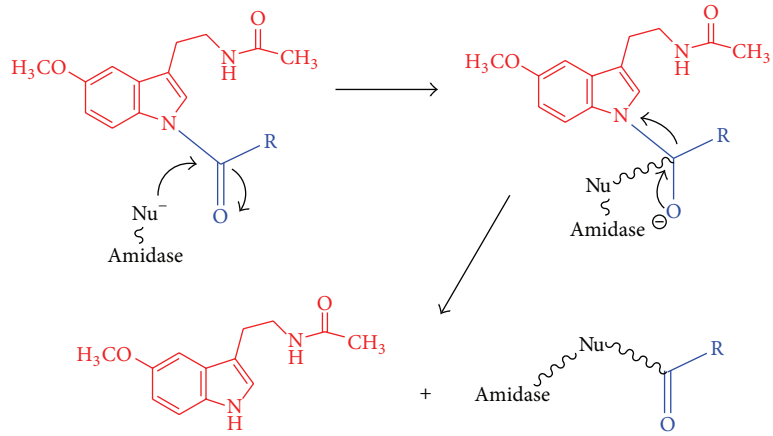


Figure 3: Illustration of amidase catalyzed release of melatonin from melatonin derivatives (Nu^- = SH, NH_2, OH, etc.).

Thus, it seemed that a proposed release mechanism for melatonin, catalyzed by amidases (Figure 3), was not efficient enough.

Compound **10** appeared to have much better melatonin releasing profile, with melatonin release rates achieving 26.9± 2.1, 44.7 ± 3.2, and 44.7 ± 3.2% after 2-, 3-, and 4-hour-incubation. Especially, compounds **11** and **12** showed the most impressive melatonin release rates. After 2-hour incubation time, 37.8 ± 2.9 and 44.7 ± 3.1% of melatonin were released from compounds **11** and **12**, respectively. These data after 3-hour incubation time were 67.3 ± 3.5, and 67.3 ± 3.5%; and after 3-hour incubation were 75.2 ± 3.8 and 83.5 ± 4.2, respectively (Table 1). The more efficient release of melatonin from compounds **11** and **12** could be speculated, in part, by the self-immolation assisted hydrolysis (Figure 4). This property has been commonly observed with succinyl and glutaroyl prodrugs and other similar ones [18–20]. From these results, it is expected that compounds **11** and **12** could serve as promising sustained release prodrugs of melatonin for using as natural-sleep inducers.

3. Conclusion

We have designed and synthesized twelve melatonin amide prodrugs aiming at prolonging the action of melatonin

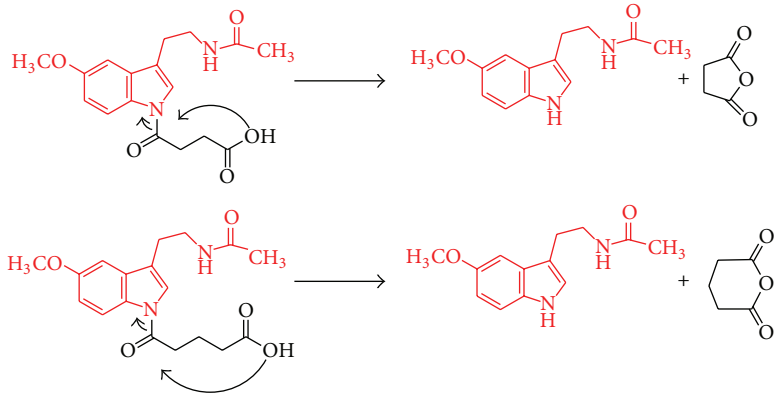


Figure 4: Illustration of self-immolation release of melatonin from compounds **11** and **12**.

in vivo by improving its half-life. The N^1-succinyl and N^1-glutaroylmelatonin derivatives (compounds **11** and **12**) could be promising as sustained release melatonin prodrugs.

4. Experimental Protocols

All products were homogenous, as examined by thin-layer chromatography (TLC), performed on Whatman 250 μm Silica Gel GF Uniplates and visualized under UV light at 254 and 365 nm. Melting points were determined with an Electrothermal Melting Point apparatus and are uncorrected. Chromatographic purification was done by the open flash silica gel column chromatography using Merck silica gel 60 (240 to 400 mesh). Nuclear magnetic resonance spectra (^{1}H-NMR) were recorded using tetramethylsilane as an internal standard on a Bruker 500 MHz spectrometer with DMSO-d_6 as solvent unless otherwise indicated. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane as internal standard. Electron ionization (EI), electrospray ionization (ESI), and high-resolution mass spectra were obtained using PE Biosystems API 2000 and Mariner mass spectrometers, respectively. Reagents and solvents were purchased from Aldrich or Fluka Chemical Corp. (Milwaukee, WI, USA) or Merck unless noted otherwise. Solvents were distilled and dried before use. Elemental analysis results were found within ±0.3%.

General Procedures for Synthesis of Compounds ***1–10***. In a 50 mL round bottom flask, sodium hydride was placed (120 mg, 3 mmoL, 60% in oil). Approximately 3 mL of n-hexane (anhydrous) was added. After gentle shaking, the n-hexane layer was discarded. A solution of melatonin (0.46 g, 2 mmoL) dissolved in 5 mL of dimethylformamide (DMF) was added in portion to the reaction flask. The resulting mixture was stirred at room temperature for 30 minutes then cooled to 0–5°C in an ice bath. Then, acyl chloride (3 mmoL) was dropwise added over 10–15 minutes into the reaction mixture to maintain the temperature in the reaction flask below 5°C. After the addition was complete, the temperature of the reaction mixture was raised to about 80–100°C, and stirring was continued for about 24 hours. After that, the reaction mixture was cooled and slowly transferred into a cold solution of sodium bicarbonate (10%, 100 mL). Precipitates were collected and recrystallized from n-hexane-acetone to furnish the target compounds **1–10**.

N-(2-(1-Acetyl-5-methoxy-1H-indol-3-yl)ethyl)acetamide ***(1)***. White solid; Yield: 87%; Mp: 144–147°C; IR (KBr, cm^{-1}): 3325 (NH), 3020 (CH-aromatic), 2930, 2910 (CH_3, CH_2), 1715, 1680 (C=O), 1618, 1583, 1481 (C=C, aromatic), 1250 (C–O). ESI-MS (m/z): 275 $[M + H]^+$, 232 $[M–CH_3CO]^+$, 190 $[M–2CH_3CO]^+$, 160 $[M–2CH_3CO–OCH_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.36 (s, 1H), 7.09 (*d*, 1H, J = 8.20 Hz), 6.67 (1H, *d*, J = 8.20 Hz), 6.63 (s, 1H), 3.71 (s, 3H), 3.46–2.66 (4H, m), 2.10 (s, 3H), 2.07 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6, ppm): δ 169.89, 167.07, 157.65, 131.24, 128.52, 125.72, 113.32, 110.43, 109.23, 104.32, 57.80, 24.31, 23.25. Anal. $C_{15}H_{18}N_2O_3$: C, H, N.

Synthesis of N-(2-(5-Methoxy-1-propionyl-1H-indol-3-yl)ethyl) hyl)acetamide ***(2)***. White solid; Yield: 80%; Mp: 152–154°C; IR (KBr, cm^{-1}): 3330 (NH), 3015 (CH-aromatic), 2925, 2910 (CH_3, CH_2), 1720, 1680 (C=O), 1620, 1580, 1475 (C=C, aromatic), 1251 (C–O). ESI-MS (m/z): 289 $[M + H]^+$, 246 $[M–CH_3CO]^+$, 190 $[M–CH_3CO–C_2H_5CO]^+$, 160 $[M–CH_3CO–C_2H_5CO–OCH_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.46 (s, 1H), 7.22 (*d*, 1H, J = 8.25 Hz), 6.71 (1H, *d*, J = 8.25 Hz), 6.69 (s, 1H), 3.82 (s, 3H), 3.47–2.69 (4H, m), 2.47 (2H, m), 2.15 (s, 3H), 2.11 (m, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6, ppm): δ 170.45, 169.45, 156.34, 130.34, 129.42, 126,46, 112.32, 111.78, 110.32, 103.38, 56.43, 28.67, 24.31, 15.47. Anal. $C_{16}H_{20}N_2O_3$: C, H, N.

Synthesis of N-(2-(1-Butyryl-5-methoxy-1H-indol-3-yl)ethyl) acetamide ***(3)***. White solid; Yield: 84%; Mp: 138–141°C; IR (KBr, cm^{-1}): 3330 (NH), 3015 (CH-aromatic), 2925, 2910 (CH_3, CH_2), 1710, 1670 (C=O), 1620, 1580, 1475 (C=C, aromatic), 1251 (C–O). ESI-MS (m/z): 303 $[M + H]^+$, 260 $[M–CH_3CO]^+$, 190 $[M–CH_3CO–C_3H_7CO]^+$, 160 $[M–CH_3CO–C_3H_7CO–OCH_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.39 (s, 1H), 7.17 (*d*, 1H, J = 7.89 Hz), 6.73 (1H, *d*, J = 7.89 Hz), 6.65 (s, 1H), 3.77 (s, 3H), 3.41–2.60 (4H, m), 2.45 (2H, m), 2.06 (m, 3H), 1.63 (m, 2H), 0.99 (m, 3H).

*Synthesis of N-(2-(5-Methoxy-1-pentanoyl-1H-indol-3-yl)ethyl) acetamide (**4**)*. White solids; Yield: 75%; Mp: 157–160°C; IR (KBr, cm^{-1}): 3215 (NH), 3020 (CH-aromatic), 2930, 2915 (CH_3, CH_2), 1700, 1675 (C=O), 1610, 1572, 1479 (C=C, aromatic), 1251, 1210. ESI-MS (m/z): 321 $[M + H]^+$, 274 $[M–CH_3CO]^+$, 190 $[M–CH_3CO–C_4H_9CO]^+$, 160 $[M–CH_3CO–C_4H_9CO–OCH_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.41 (s, 1H), 7.13 (*d*, 1H, J = 7.90 Hz), 6.71 (1H, *d*, J = 7.90 Hz), 6.69 (s, 1H), 3.79 (s, 3H), 3.45–2.63 (4H, m), 2.45 (2H, m), 2.10 (m, 3H), 1.64–1.37 (m, 4H), 0.97 (m, 3H).

*Synthesis of N-(2-(1-Hexanoyl-5-methoxy-1H-indol-3-yl)ethyl) acetamide (**5**)*. White solid; Yield: 79%; Mp: 147–149°C; IR (KBr, cm^{-1}): 3227 (NH), 3030 (CH-aromatic), 2950, 2915, 2910 (CH_3, CH_2), 1695, 1675 (C=O), 1630, 1590, 1470 (C=C, aromatic), 1250 (C–O). ESI-MS (m/z): 335 $[M + H]^+$, 288 $[M–CH_3CO]^+$, 190 $[M–CH_3CO–C_5H_{11}CO]^+$, 160 $[M–CH_3CO–C_5H_{11}CO–OCH_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.39 (s, 1H), 7.11 (*d*, 1H, J = 7.80 Hz), 6.75 (1H, *d*, J = 7.80 Hz), 6.73 (s, 1H), 3.81 (s, 3H), 3.47–2.66 (4H, m), 2.47 (2H, m), 2.11 (m, 3H), 1.65–1.33 (m, 6H), 0.96 (m, 3H).

*Synthesis of N-(2-(1-Heptanoyl-5-methoxy-1H-indol-3-yl)ethyl) acetamide (**6**)*. White solid; Yield: 68%; Mp: 146–148°C; IR (KBr, cm^{-1}): 3310 (NH), 3020 (CH-aromatic), 2905, 2900 (CH_3, CH_2), 1710, 1670 (C=O), 1600, 1574, 1482 (C=C, aromatic), 1253 (C–O). ESI-MS (m/z): 349 $[M + H]^+$, 302 $[M–CH_3CO]^+$, 190 $[M–CH_3CO–C_6H_{13}CO]^+$, 160 $[M–CH_3CO–C_6H_{13}CO–OCH_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.36 (s, 1H), 7.09 (*d*, 1H, J = 8.12 Hz), 6.79 (1H, *d*, J = 8.12 Hz), 6.71 (s, 1H), 3.74 (s, 3H), 3.41–2,62 (4H, m), 2.46 (2H, m), 2.04 (m, 3H), 1.62–1.34 (m, 8H), 0.98 (m, 3H).

*Synthesis of N-(2-(5-Methoxy-1-octanoyl-1H-indol-3-yl)ethyl) acetamide (**7**)*. White solid; Yield: 71%; Mp: 140–142°C; IR (KBr, cm^{-1}): 3150 (NH), 3003 (CH-aromatic), 2950, 2925 (CH_3, CH_2), 1690, 1665 (C=O), 1620, 1570, 1480 (C=C, aromatic), 1250, 1210 (C–O). ESI-MS (m/z): 363 $[M + H]^+$, 316 $[M–CH_3CO]^+$, 190 $[M–CH_3CO–C_7H_{15}CO]^+$, 160 $[M–CH_3CO–C_7H_{15}CO–OCH_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.41 (s, 1H), 7.11 (*d*, 1H, J = 8.11 Hz), 6.83 (1H, *d*, J = 8.11 Hz), 6.74 (s, 1H), 3.77 (s, 3H), 3.44–2.65 (4H, m), 2.47 (2H, m), 2.09 (m, 3H), 1.67–1.33 (m, 10H), 0.99 (m, 3H).

*Synthesis of N-(2-(5-Methoxy-1-nonanoyl-1H-indol-3-yl)ethyl) acetamide (**8**)*. White solid; Yield: 65%; Mp: 139–143°C; IR (KBr, cm^{-1}): 3225 (NH), 3023 (CH-aromatic), 2945, 2915, 2910 (CH_3, CH_2), 1695, 1672 (C=O), 1630, 1590, 1470, 1250 (C–O). ESI-MS (m/z): 377 $[M + H]^+$, 330 $[M–CH_3CO]^+$, 190 $[M–CH_3CO–C_8H_{17}CO]^+$, 160 $[M–CH_3CO–C_8H_{17}CO–OCH_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.47 (s, 1H), 7.24 (*d*, 1H, J = 8.32 Hz), 6.89 (1H, *d*, J = 8.32 Hz), 6.79 (s, 1H), 3.91 (s, 3H), 3.51–2.69 (4H, m), 2.52 (2H, m), 2.12 (m, 3H), 1.69–1.30 (m, 12H), 1.00 (m, 3H).

*Synthesis of N-(2-(1-Decanoyl-5-methoxy-1H-indol-3-yl)ethyl) acetamide (**9**)*. White solid; Yield: 63%; Mp: 155–158°C; IR (KBr, cm^{-1}): 3240 (NH), 3020 (CH-aromatic), 2945, 2935, 2915 (CH_3, CH_2), 1710, 1670 (C=O), 1620, 1580, 1485 (C=C, aromatic), 1250. ESI-MS (m/z): 391 $[M + H]^+$, 344 $[M–CH_3CO]^+$, 190 $[M–CH_3CO–C_9H_{19}CO]^+$, 160 $[M–CH_3CO–C_9H_{19}CO–OCH_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.42 (s, 1H), 7.21 (*d*, 1H, J = 8.29 Hz), 6.92 (1H, *d*, J = 8.29 Hz), 6.81 (s, 1H), 3.99 (s, 3H), 3.52–2.71 (4H, m), 2.57 (2H, m), 2.10 (m, 3H), 1.71–1.32 (m, 14H), 1.02 (m, 3H).

*Synthesis of Isobutyl 3-(2-Acetamidoethyl)-5-methoxy-1H-indole-1-carboxylate (**10**)*. White solid; Yield: 75%; Mp: 167–169°C; IR (KBr, cm^{-1}): 3250 (NH), 3050 (CH-aromatic), 2930, 2910 (CH_3, CH_2), 16950, 1660 (C=O), 1610, 1545, 1480 (C=C, aromatic), 1220 (C–O). ESI-MS (m/z): 333 $[M + H]^+$, 290 $[M–CH_3CO]^+$, 190 $[M–CH_3CO–C_4H_9CO_2]^+$, 160 $[M–CH_3CO–C_4H_9CO_2–OCH_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.35 (s, 1H), 7.15 (*d*, 1H, J = 8.50 Hz), 6.95 (1H, *d*, J = 8.50 Hz), 6.82 (s, 1H), 4.09–4.01 (2H, m), 3.81 (s, 3H), 3.47–3.41 (m, 2H), 2.65–2.45 (m, 4H), 2.10 (m, 3H), 1.07–1.01 (m, 6H). Anal. $C_{18}H_{24}N_2O_4$: C, H, N.

*Synthesis of 4-(3-(2-Acetamidoethyl)-5-methoxy-1H-indol-1-yl)-4-oxobutanoic Acid (**11**)*. In a 50 mL round bottom flask sodium hydride was placed (120 mg, 3 mmoL, 60% in oil). Approximately 3 mL of n-hexane (anhydrous) was added. After gentle shaking, the n-hexane layer was discarded. A solution of melatonin (0.46 g, 2 mmoL) dissolved in 5 mL of dimethylformamide (DMF) was added in portion to the reaction flask. The resulting mixture was stirred at room temperature for 30 minutes then cooled to 0–5°C in an ice-bath. Then, succinic anhydride (2.2 mmoL) was added in portions into the reaction mixture. After the addition was complete, the temperature of the reaction mixture was raised to about 80–100°C, and stirring was continued for about 24 hours. After that, the reaction mixture was cooled and slowly transferred into 50 mL of crushed-ice water. pH was adjusted to 2 by a solution of 5% HCl. Precipitates were collected and recrystallized from ethanol to furnish the target compound.

White solid; Yield: 66%; Mp: 166–170°C; IR (KBr, cm^{-1}): 3450, 3300 (OH), 3220 (NH), 3033 (CH-aromatic), 2940, 2930, 2910 (CH_3, CH_2), 1725, 1680, 1675 (C=O), 1615, 1580, 1485 (C=C, aromatic), 1250 (C–O). ESI-MS (m/z): 333 [M + H], 290 $[M–CH_3CO]$, 246 $[M–CH_3CO–COOH]$, 190 $(M–CH_3CO–COCH_2CH_2COOH)$, 160 $[M–CH_3CO–COCH_2CH_2COOH–OCH_3]$. ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.39 (s, 1H), 7.11 (*d*, 1H, J = 8.21 Hz), 6.71 (1H, *d*,J = 8.21 Hz), 6.67 (s, 1H), 3.88 (s, 3H), 3.47–2.63 (4H, m), 2.67–2.57 (m, 4H), 2.11 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6, ppm): δ 175.90, 170.31, 169.21, 155.35, 130.24, 129.84, 126.42, 112.42, 109.32, 108.12, 103.45, 56.89, 42.28, 34.89, 32.78, 28.77, 24.23. Anal. $C_{17}H_{20}N_2O_5$: C, H, N.

*Synthesis of 5-(3-(2-Acetamidoethyl)-5-methoxy-1H-indol-1-yl)-5-oxopentanoic Acid (**12**).* This compound was synthesized by a similar procedure used for the synthesis of compound **11** but using glutaric anhydride instead of succinic anhydride.

White solid; Yield: 64%; Mp: 163–164°C; IR (KBr, cm^{-1}): 3440, 3320, 2935, 2925, 2915 (CH_3, CH_2), 1720, 1685 (C=O), 1620, 1615, 1587, 1482 (C=C, aromatic), 1255 (C–O). ESI-MS (m/z): 34 $[M + H]^+$, 304 $[M–CH_3CO]^+$, 190 $[M–CH_3CO–COCH_2CH_2CH_2COOH]^+$, 160 $[M–CH_3CO–COCH_2CH_2CH_2COOH–OCH_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.43 (s, 1H), 7.17 (*d*, 1H, J = 8.23 Hz), 6.75 (1H, *d*, J = 8.23 Hz), 6.69 (s, 1H), 3.89 (s, 3H), 3.49–2.66 (4H, m), 2.49–2.21 (m, 6H), 2.10 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6, ppm): δ 176.80, 169.67, 168.43, 156.85, 129.90, 128.44, 127.42, 111.32, 110.92, 107.87, 104.65, 57.65, 42.25, 36.55, 32.66, 29.66, 24.33, 21.78. Anal. $C_{18}H_{22}N_2O_5$: C, H, N.

Determination of the Hydrolysis Percentages of Compounds. The hydrolysis percentage of each compound in an 80% human plasma (pH 7.4) was determined using previously reported method [16]. Briefly, a solution of 20 mg of melatonin or its derivatives was prepared in acetonitrile (5 mL) and was added to 95 mL of 80% human plasma (pH 7.4, prepared by mixing plasma with phosphate buffer pH 7.4 in a ratio of 4 to 1). An aliquot of 10 mL of this solution was withdrawn and kept in test tubes maintained at 37 ± 0.5°C. After each interval of time (1, 2, 3, and 4 hours) an aliquot of 500 μL was withdrawn from each tube and transferred to a microcentrifuge tube (Eppendorf's tube). The tubes were placed in freezing mixture in order to stop any further hydrolysis, followed by vortexing thoroughly for 5 min. After vortexing, the tubes were centrifuged at high speed (10000 rpm) for 5 min. A clear supernatant (20 mL) obtained from each tube was used for HPLC analysis with ODS C18 column, mobile phase including acetonitrile and water (45 : 65), and UV detector set at 257 nm wavelength to detect peaks of melatonin and its derivatives. Compounds concentration and percentage in a mixture were quantified by peak area calculation and analysis.

Conflict of Interests

The authors declare no conflict of interests.

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