Novel Melatonin Derivatives: Synthesis, Anticancer Evaluations and Molecular-Docking Study

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†Blessing and mercy to the soul of our dear fellow (late) Mervat M. AbdElhalim

Abstract

Many studies mentioned that Melatonin considers an anti-cancer agent. So in this study, a lot of novel Melatonin derivatives incorporated different heterocyclic ring systems such as triazole, thiadiazole, tetrazole, thiazole, thiophene and pyrazole were synthesized. The synthesized compounds 5, 6, 8, 11, 15, 16 and 19 were evaluated as anti-cancer by using two human cancer cell lines, Breast cancer (MCF7) and colon cancer (HCT-116). The synthesized compounds showed a gradual decrease in the cell viability of the two cell lines. We also observed that compound 16 was the lowest IC50 and the highest cytotoxic effects against the two cancer cell lines. Furthermore, the molecular-docking study was employed to determine the possible mode of action of the synthesized compounds against proteins (CDK2 and P53-MDM2) which, were considered to be potential proteins involved in the pathogenesis of cancer. We observed that compound 16 was the best-docked ligand against the targeted proteins, as it displayed the lowest binding energies, critical hydrogen bonds, and hydrophobic interactions compared to other tested compounds.

Keywords: Anticancer; azole, azine; thiophene; Melatonin; molecular-docking

Introduction

Melatonin is a natural hormone mainly produced by the pineal gland that controls sleep awakening cycles [1]. It is a common molecule and widely found in nature with multifunctional activity occurring in unicellular organisms, plants, fungi, and all mammals [2]. Melatonin was biosynthesized from tryptophan by 4 step procedure, firstly tryptophan was hydroxylated to 5-hydroxytryptophan then decarboxylated with the formation of serotonin. Serotonin is acetylated to N-acetylserotonin; this product was methylated to give Melatonin [3-5]. Past research showed that Melatonin had multifunctional effects as an antioxidant [6,7], analgesic [8], immunomodulatory [9,10], cardiovascular disorder [11], stroke protective [12], treatment of chronic renal disease [13], neuro-protective [14] and anticancer effect [15]. Several clinical studies investigated the therapeutic value of Melatonin in different types of cancer. Also, derivatives of Melatonin had very low toxicity over a wide range of doses [16]. We began our research from here and synthesized new heterocycles derivatives using Melatonin as a starting compound. These derivatives screened for their anticancer activity against human cancer breast and colon cell lines.

Moreover, there are some important effectiveness of Melatonin for high blood pressure, anxiety before surgery, sunburn, temporomandibular disorders (TMD), and thrombocytopenia. Also, some research papers indicated that Melatonin can get a better sleeping, problem relative to conditions such as schizophrenia [17], autism [18], epilepsy [19], depression [20], intellectual disabilities [21], and developmental disabilities [22]. Furthermore, due to the great medicinal importance of Melatonin, some of the recently published research discussed the benefits of Melatonin in the attenuation and as a potential adjuvant treatment of COVID-19 [23-25]. On the
other hand, the resultant heterocyclic ring systems had a great attention due to their biologically active effect especially as antimicrobial [26,27], antiviral [28,29], anti-tubercular [30,31], antioxidant [32,33], anti-inflammatory [34,35], anti-depressant [36,37], anti-mitotic [38,39], kinases inhibiting [40,41] and anti-cancer [42,43].

In this study, we focused on the synthesized of new Melatonin derivatives via a combination of melatonin molecule with heterocyclic moiety possessing appropriate anticancer activity against human breast cancer (MCF7) and colon cancer (HCT116) cell lines. Also, the molecular docking study was carried out for some selected biologically active synthesized compounds that are consistent with the in vitro activity.

Experimental

Synthetic methods, analytical and spectral data

The starting hormone (Melatonin) was purchased from Sigma Company, USA. All solvents were anhydrated by distillation prior to use. All melting points were measured by using an Electrothermal Engineering Ltd. (Cat No. IA9100 MK3, 400 C Max) apparatus and were uncorrected. The IR spectra were recorded in KBr discs on a Shimadzu FT-IR 8201 PC spectrophotometer and expressed in per centimeter. The 1H NMR and 13C NMR spectra were recorded with Bruker at 400 and 100 MHz, respectively, in DMSO-d6 and dry aceton as solvent, and chemical shifts were recorded in parts per million relative to TMS. The spin multiplicities were abbreviated as follows: s—singlet, d— doublet, t—triplet, q— quartet, and m (multiplet, more than quartet). Mass spectra were recorded on a GCMS-QP 1000 EX mass spectrometer operating at 70 eV. Elemental analyses were carried by the Micro analytical Data Unit at Cairo University, Giza, Egypt. The reactions were monitored by thin layer chromatography (TLC) which was carried out using Merck 60 F254 aluminum sheets and visualized by UV light (254 nm). The mixtures were separated by preparative TLC and gravity chromatography.

Synthesis of ethyl 2-(3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl)acetate (2)

To a solution of Melatonin (0.93 g, 4 mmol) in dimethylformamide (15 mL), grinded KOH (0.22 g, 4 mmol) and ethyl chloroacetate (0.49 g, 4 mmol) were added and the reaction mixture heated under reflux for 8 hours until all the starting materials disappeared as indicated by TLC. A gummy solid product formed upon pouring onto an ice/water mixture and neutralized by 0.1 N hydrochloric acid. The formed solid product was collected by filtration and crystallized from ethanol. Off white crystals, yield (1.0 g, 82%), mp (127-130 °C. IR (KBr, cm⁻¹): ν 3300 (NH), 3050 (CH-aromatic), 2980, 2891 (CH₃, CH₂), 1720, 1655 (2 C=O), 1580, 1451 (C=C). 1H NMR (Dry acetone, ppm) δ: 1.30 (t, J = 7.48 Hz, 3H, CH₃), 1.88 (s, 3H, COCH₃), 2.89 (t, J = 7.20 Hz, 2H, CH₂), 3.46 (t, J = 6.76 Hz, 2H, CH₂), 3.82 (s, 3H, OCH₃), 4.14 (q, J = 7.48 Hz, 2H, CH₂), 4.18 (s, 2H, CH₂), 6.74-7.28 (m, J = 8.72 Hz, 5H, C₆H₅, NH, pyrrole-H). Elemental analysis for C₁₇H₂₅N₂O₃ (318.37), (% Calculated/Found): C, 64.13/63.90; H, 6.97/6.72; N, 8.80/8.60.

Synthesis of N-(2-(1-(2-hydrazinyl-2-oxoethyl)-5-methoxy-1H-indol-3-yl)methyl)acetamide (3)

A mixture of compound 2 (1.27 g, 4 mmol) and hydrazine hydrate (0.6 mL, 8 mmol) in absolute ethanol (20 mL) was heated under reflux 6 hrs, until all the starting materials disappeared as indicated by TLC. The reaction mixture was concentrated then cooling. The formed solid product was collected and crystallized from absolute ethanol. Pale yellow crystals, yield (0.90 g, 98.6%), mp (127-130 °C). IR (KBr, cm⁻¹): ν 3305 (NH), 3078 (CH-aromatic), 2989, 2893 (CH₃, CH₂), 1725, 1672 (2 C=O), 1585, 1455 (C=C). 1H NMR (Dry Acetone, ppm) δ: 1.90 (s, 3H, COCH₃), 2.74 (t, J = 7.24 Hz, 2H, CH₂), 2.90 (s, 2H, CH₂), 3.30 (t, J = 7.40 Hz, 2H, CH₂), 3.66 (s, 3H, OCH₃), 6.59-7.13 (m, J = 8.76 Hz, 8H, C₆H₅; pyrrole-H; 2NH; NH₂). Elemental analysis for C₁₅H₂₆N₄O₃ (304.34), (% Calculated/Found): C, 59.20/59.40; H, 6.62/6.33; N, 18.41/18.20.

Synthesis of N-(2-(5-methoxy-1-(2-oxo-2-(phenylcarbamothioyl)hydrazinyl)ethyl)-1H-indol-3-yl)ethylacetamide (4)

A mixture of compound 3 (1.26 g, 4 mmol) and phenylisothiocyanate (0.54 g, 4 mmol) in absolute ethanol (15 mL) was heated under reflux for 2 hrs, until all the starting materials disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture. The formed solid product was collected by filtration and crystallized from absolute ethanol. Yellow crystals, yield (0.32 g, 28.68%), mp (114-116 °C. IR (KBr, cm⁻¹): ν 3206 (NH), 3113 (NH), 2935, 2854 (CH₃, CH₂), 1685, 1680 (2 C=O), 1597, 1454 (C=C), 1337, 1242 (C=S). 1H NMR (Dry Acetone, ppm) δ: 1.88 (s, 3H, COCH₃), 3.00 (m, 4H, 2CH₂), 3.62 (s, 2H, CH₂), 3.82 (s, 3H, OCH₃), 7.12-7.69 (m, J = 7.84 Hz, 13H, C₆H₅; 4NH; pyrrole-H). Elemental analysis for C₂₈H₃₀N₆O₈S (439.53), (% Calculated/Found): C, 60.12/60.30; H, 5.73/5.90; N 15.93/15.70; S, 7.30/7.01.

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Synthesis of N-(2-(1-((5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)methyl)-5-methoxy-1H-indol-3-yl)ethyl)acetamide (5)

A solution of compound 4 (0.21 g, 0.47 mmol) in sodium hydroxide ethanolic (4 N, 50 ml) refluxed in water bath for 4 hrs, concentrated, cooling, then adjust pH between 5-6 with HCl then leave it to 1 hr then filter off and crystallized from absolute ethanol. Brown crystals, yield (0.13 g, 65.69%), mp 208-210 °C. IR (KBr, cm−1): ν 3213 (NH), 2928, 2854 (CH3, CH2), 1600 (C=O), 1545, 1445 (C=C). 1H NMR (Dry acetone, ppm): δ: 1.90 (s, 3H, COCH3), 2.69 (m, 4H, 2CH2), 3.50 (s, 3H, OCH3), 6.82-6.85 (s, 3H, CH2, pyrrole-H), 7.05-7.55 (m, J = 7.80 Hz, 8H, C6H5, C6H3), 8.81 (s, 1H, NH), 9.59 (s, 1H, SH). MS (EI) m/z (%): 423.56 [M+2] (0.09), 421.57 [M] (0.09), 420.32 [M+1] (0.05), 161.02 (100.00), 77.01 [C6H4]1 (61.71). Elemental analysis for C21H15N2O3S: (421.52), (% Calculated/Found): C, 62.69/62.93; H, 5.50/5.20; N, 16.61/16.30; S, 7.61/7.92.

Synthesis of N-(2-(5-methoxy-1-((5-phenylamino)-1,3,4-thiadiazol-2-yl)methyl)-1H-indol-3-yl)ethyl)acetamide (6)

A solution of compound 4 (1.79 g, 4 mmol) was added gradually with stirring to cold conc. H2SO4 (10 mL) during 10 min. The mixture was further stirred for another 1 h in an ice bath. Then the mixture was poured over crushed ice with stirring. The solid separated out was filtered, washed with water, and crystallized with ethanol. Off white crystals, yield (0.12 g, 60.64%), mp (151-154) °C. IR (KBr, cm−1): ν 3213 (NH), 2981, 2897 (CH3, CH2), 1680 (C=O), 1597, 1473 (C=C). 1H NMR (Dry acetone, ppm): δ: 1.90 (s, 3H, COCH3), 3.18 (m, 4H, 2CH2), 3.60 (s, 3H, OCH3), 6.89-6.92 (s, 3H, CH2, pyrrole-H), 7.04-7.55 (m, m, J = 7.76 Hz, 8H, C6H5, C6H3), 9.61 (s, 1H, NH), 12.30 (s, 1H, NH). 13C NMR (DMSO-d6, ppm) δ: 39.4 (2), 160.4, 117.5 (2), 117.9 (2), 121.8, 122.7, -129.5 (2), 129.6 (2), 140.2, 141.4, 156.3 (2), 156.9, 181.7. Elemental analysis for C21H15N2O3S: (421.52), (% Calculated/Found): C, 62.69/62.91; H, 5.50/5.73; N, 16.61/16.30; S, 7.61/7.33.

Synthesis of N-(2-(1-(cyanomethyl)-5-methoxy-1H-indol-3-yl)ethyl)acetamide (7)

To a mixture of Melatonin (0.93 g, 4 mmol) and anhydrous potassium carbonate (0.55 g, 4 mmol) were dissolved in dimethylformamide (20 mL) and the solution was stirred for 1 hr. chloroacetanilide (0.25 mL) was added and the reaction mixture was stirred for 12 hrs until all the starting materials disappeared as indicated by TLC. The reaction mixture treated with ice/water mixture, then extracted by chloroform (5.20 mL), dried over anhydrous sodium sulphate and filtered off then evaporated. The formed solid product was collected and crystallized from absolute ethanol. Yellow crystals, yield (0.95 g, 70.1%), mp (186-189) °C. IR (KBr, cm−1): ν 3302 (NH), 2989, 2897 (CH3, CH2), 1720, 1680 (2 C=O),

Synthesis of N-(2-(1-((2H-tetrazol-5-yl)methyl)-5-methoxy-1H-indol-3-yl)methyl)ethyl)acetamide (8)

To a mixture of compound 7 (1.09 g, 4 mmol), sodium azide (0.26 g, 4 mmol) and ammonium chloride (0.21 g, 4 mmol) in dimethylformamide (10 mL) was added and heated under reflux for 24 hrs, until all the starting materials disappeared as indicated by TLC then diluted with cold water (20 mL). The reaction extracted by chloroform (5.20 mL), dried over anhydrous sodium sulphate and filtered off then evaporated. The formed solid product was collected and crystallized from absolute ethanol. Pale yellow crystals, yield (0.90 g, 86.85%), mp (105-108) °C. IR (KBr, cm−1): ν 3302 (NH), 2989, 2897 (CH3, CH2), 1680 (C=O), 1620, 1489 (C=C), 1550 (C=N). 1H NMR (Dry acetone, ppm): δ: 1.89 (s, 3H, COCH3), 2.90 (t, J = 7.52 Hz, 2H, CH2), 3.05 (s, 2H, CH2), 3.48 (t, J = 7.52 Hz, 2H, CH2), 3.82 (s, 3H, OCH3), 6.74-7.29 (m, J = 8.76 Hz, 5H, C6H5, pyrrole-H, NH), 9.96 (s, 1H, NH). 13C NMR (DMSO-d6, ppm) δ: 23.2, 25.8, 40.6, 55.8 (2), 100.6, 111.5, 112.2, 112.5, 128.1 (2), 123.7, 131.9, 153.5, 169.6. MS (EI) m/z (%): 315.25 [M+1] (4.94), 314.15 [M] (12.24), 75.05 (100.00). Elemental analysis for C15H15N2O2 (314.34), (% Calculated/Found): C, 57.31/57.60; H, 5.77/5.98; N, 26.74/26.60.

Synthesis of ethyl 2-5-((3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl)methyl)-2H-tetrazol-2-yl)acetate (9)

Mixture of compound 8 (1.28 g, 4 mmol), ethyl chloroacetate (0.49 g, 4 mmol) and anhydrous potassium carbonate (0.55 g, 4 mmol) in dry acetone (20 mL) was refluxed for 22 hrs, until all the starting materials disappeared as indicated by TLC. The reaction mixture was evaporated, the residue was dissolved in water, the produced was extracted with ether, dried over sodium sulphate and filtered off, then evaporated and crystallized. The formed solid product was collected and crystallized from absolute ethanol. Yellow crystals, yield (0.75 g, 75%), mp (110-113) °C. IR (KBr, cm−1): ν 3302 (NH), 2989, 2897 (CH3, CH2), 1720, 1680 (2 C=O),
1620, 1480 (C=C), 1550 (C=N). 1H NMR (Dry acetone, ppm) δ: 1.13 (t, J = 8.0 Hz, 3H, CH₃), 1.89 (s, 3H, COCH₃), 2.75 (t, J = 7.24 Hz, 2H, CH₂), 3.34 (t, 2H, CHO), 3.36 (s, 4H, 2CH₂O), 3.66 (s, 3H, OCH₃), 4.13 (q, J = 8.00 Hz, 2H, CH₂), 6.59-7.12 (m, J = 8.72 Hz., 4H, C₆H₄, pyrrole-H). 9.76 (s, 1H, NH). MS (EI) m/z (%) : 475.20 [M⁺]+ [0.11%], 161.05 (100.00), 77.08 [C₄H₈]⁺ (49.27). Elemental analysis for C₁₉H₁₄N₃O₃ (474.52), (% Calculated/Found): C, 60.7560/50; H, 5.52/5.30; N, 23.61/23.81.

Synthesis of N-(2-(1-(2-(2-hydrazinyl-2-oxoethyl)-2H-tetrazol-5-yl)methyl)-5-methoxy-1H-indol-3-yl)(ethyl)acetamide (10)

Mixture of compound 9 (1.60 g, 4 mmol), in ethanol absolute (20 mL) and hydrazine hydrate (0.6 mL, 8 mmol) was refluxed for 6 hrs until all the starting materials disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture, the produced was extracted with ether, dried over sodium sulphate anhydrous and filtered off, then evaporated and crystallized. The solid product was collected and crystallized from absolute ethanol. Off white crystals, yield (0.60 g, 86.3%), mp 102-105 °C. IR (KBr, cm⁻¹): ν 3302 (NH), 2989, 2897 (CH₃, CH₂), 1685, 1680 (2 C=O), 1627, 1489 (C=C), 1550 (C=N). 1H NMR (Dry acetone, ppm) δ: 1.89 (s, 3H, COCH₃), 2.75 (t, J = 7.28 Hz, 2H, CH₂), 3.34 (t, J = 7.36 Hz 2H, CH₂), 3.36 (s, 2H, CH₂) 3.66 (s, 3H, OCH₃), 6.60-6.62 (s, 3H, CH₂, pyrrole-H), 6.97-7.13 (m, J = 8.72 Hz, 6H, C₆H₄, NH₂, NH), 9.76 (s, 1H, NH). MS (EI) m/z (%): 386.20 [M⁺] [0.16%], 385.15 [M⁺-1] (0.29), 160.10 (100.00). Elemental analysis for C₁₉H₁₂N₄O₃ (386.41), (% Calculated/Found): C, 52.84/52.99; H, 5.74/5.90; N, 29.00/28.70.

Synthesis of N-(2-(1-(2-(2-benzylidenehydrazinyl)-2-oxoethyl)-2H-tetrazol-5-yl)(methyl)-5-methoxy-1H-indol-3-yl)(ethyl)acetamide (11)

To a solution of compound 10 (1.55 g, 4 mmol) in ethanol (10 mL), benzaldehyde (0.42 g, 4 mmol) and glacial acetic acid (0.5 mL) were added. The mixture was refluxed for 5 hrs and the solvent was concentrated. After cooling, the obtained solid was filtered off, dried and crystallized from ethanol. Yellow crystals, yield (0.55 g, 89.9%), mp (106-109 °C. IR (KBr, cm⁻¹): ν 3305 (NH), 2927, 2850 (CH₃, CH₂), 1686, 1680 (2 C=O), 1585, 1482 (C=C), 1550 (C=N). 1H NMR (Dry acetone, ppm) δ: 1.88 (s, 3H, COCH₃), 2.89 (t, J = 7.28 Hz, 2H, CH₂), 3.02 (s, 3H, CH₃), 3.47 (t, 2H, CH₂), 3.89 (s, 3H, OCH₃), 6.74-6.77 (s, 3H, CH₂, pyrrole-H), 7.05-7.86 (m, J = 8.68 Hz, 9H, C₆H₄, C₆H₅, NH), 8.61 (s, 1H, NH). 13C NMR (DMSO-d₆, ppm) δ: 23.2, 25.7, 40.6, 55.8 (3), 100.6, 111.5, 112.2, 112.5, 114.9, 123.7 (2), 127.1 (2), 128.0 (2), 130.1, 131.9, 153.4 (2), 160.9, 162.2, 169.5. MS (EI) m/z (%): 475.20 [M⁺]+ [0.11%], 161.05 (100.00), 77.08 [C₄H₈]⁺ (49.27). Elemental analysis for C₁₉H₁₂N₄O₃ (474.52), (% Calculated/Found): C, 60.7560/50; H, 5.52/5.30; N, 23.61/23.81.


The data were published earlier by our group [44-46].

General Procedure for the Synthesis of Compounds 13 and 14

To a solution of compound 12 (1.33 g, 4 mmol) and phenylisothiocyanate (0.55 g, 4 mmol) add crushed potassium hydroxide (0.23 g, 4 mmol) in dimethylformamide was heated for 3 hrs then add an equimolar amount of ethyl chloroacetate (0.49 g, 4 mmol) or phenacyl bromide (0.79 g, 4 mmol) the reaction mixture, in each case, was heated under reflux for 6 hrs until all starting materials had disappeared as indicated by TLC. Then the reaction left to cool at room temperature, poured onto ice and neutralized with 0.1 N hydrochloric acid. The resulting solid product in each case was collected by filtration and crystallized from the proper solvent.


Brown crystals, yield (1.0 g, 60.7%), mp (144-147 °C. IR (KBr, cm⁻¹): ν 3421, 3244 (2 NH), 3102 (CH aromatic), 2927 (CH₃, CH₂), 2200 (CN), 1720, 1622 (2 C=O), 1564, 1480 (C=C), 1520 (C=N). 1H NMR (DMSO-d₆, ppm) δ: 1.23 (t, J = 7.48 Hz, 3H, CH₃), 1.82 (s, 3H, COCH₃), 2.79 (t, J = 9.60 Hz, 2H, CH₂), 3.38 (t , J = 9.20 Hz 2H, CH₂), 3.76 (s, 2H, CH₂), 3.82 (s, 3H, OCH₃), 4.20 (q, J = 7.48 Hz, 2H, CH₂), 6.88-7.80 (m, J = 8.80 Hz, 8H, C₆H₄, C₆H₅), 8.12 (s, 1H, NH), 11.24 (s, 1H, NH). Elemental analysis for C₂₁H₁₈N₂O₂S₂ (549.66), (% Calculated/Found): C, 59.00/59.33; H, 4.95/5.20; N, 12.74/12.90; S, 11.67/11.30%.


Brown crystals, yield (0.8 g, 45.9%), mp (90-93 °C. IR (KBr, cm⁻¹): ν 3304 (NH), 2926 (CH₃, CH₂), 2199 (CN), 1670, 1627 (2 C=O), 1580, 1483 (C=C), 1551 (C=N). 1H NMR (DMSO-d₆, ppm) δ: 1.80 (s, 3H, COCH₃), 2.77 (t, J = 9.20 Hz, 2H, CH₂), 3.31 (t, J = 8.80 Hz, 2H, CH₂), 3.75 (s, 3H, OCH₃), 4.40 (s, 2H, CH₂), 6.69-7.23 (m, J = 8.40 Hz, 13H, 2

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General Procedure for the Synthesis of Compounds 15 and 16
To a solution of compound 13 or 14 (4 mmol) in absolute ethanol (30 mL), piperidine (1 mL) was added. The reaction mixture was heated under reflux for 2-3 hrs until all starting materials had disappeared as indicated by TLC. Then left to cool at room temperature, poured onto ice and neutralized with 0.1 N hydrochloric acid. The resulting solid product was collected by filtration and crystallized from the ethanol.

N-(2-(2-(Cyano(4-oxo-3-phenylthiazolidin-2-ylidene)methyl)-7-methoxy-[1,3,4]thiadiazolo[3,2-a]indol-9-yl)ethyl)acetamide (15). Brown crystals, yield (0.45 g, 59.62%), mp (84-87 °C). IR (KBr, cm⁻¹): ν 3431 (NH), 3053 (CH aromatic), 2924, 2854 (CH₃, CH₂), 2194 (CN), 1630, 1590 (C=O), 1535, 1488 (C=C). ¹H NMR (DMSO-d₆, ppm): δ: 1.90 (s, 3H, COCH₃), 2.80 (t, 2H, CH₂), 3.34 (t, 2H, CH₂), 3.82 (3H, OCH₃), 4.16 (s, 2H, CH₂-thiazolidin), 6.85-7.70 (m, 8H, C₆H₅, C₆H₃), 9.12 (s, 1H, NH). ¹³C NMR (DMSO-d₆, ppm): δ: 22.6, 25.1, 33.3, 44.4, 55.9, 61.8, 120.9 (2), 121.1 (2), 124.7 (2), 125.4, 128.9, 129.0, 129.5 (2), 129.7 (2), 148.6 (2), 153.7, 155.5, 156.7, 172.2. MS (EI) m/z (%): 502.09 [M]+-2 (0.03), 502.76 [M]+-1 (0.02), 504.05 [M]+ (0.02), 504.86 [M]++1 (0.03), 505.71 [M]++2 (0.06), 173.06 (100), 77.25 [C₆H₅]+ (42.97). Elemental analysis for C₂₉H₂₃N₂O₅S (503.60). (Calculated/Found): C, 59.62/59.85; H, 4.20/4.40; N, 13.91/13.72; S, 12.73/12.50.

N-(2-(2-(4-Amino-5-benzoyl-2-(phenylamino)thiophen-3-yl)-7-methoxy-[1,3,4]thiadiazolo[3,2-a]indol-9-yl)ethyl)acetamide (16). Dark Red crystals, yield (0.40 g, 45.84%), mp (84-87 °C). IR (KBr, cm⁻¹): ν broad 3397 (2NH, NH₂), 3057 (CH aromatic), 2921, 2852 (CH₃, CH₂), 1645, 1617 (2 ≈ C=O), 1581, 1476 (C=C), 1552 (C=N). ¹H NMR (DMSO-d₆, ppm): δ: 1.82 (s, 3H, COCH₃) 2.78 (t, J= 8.00 Hz, 2H, CH₂), 3.34 (t, 2H, CH₂), 3.81 (3H, OCH₃), 7.03 (s, 2H, NH₂), 7.14-7.62 (m, J= 8.00 Hz, 13H, C₆H₅, C₆H₃), 11.20, 12.20 (2s, 2H, NH₂). ¹³C NMR (DMSO-d₆, ppm): δ: 23.7, 25.5, 44.3, 55.8, 101.8, 101.9, 112.8 (2), 113.1, 117.9 (2), 121.2, 121.5, 128.6, 128.9 (2), 129.3, 129.5 (2), 129.8 (2), 131.5, 133.4, 134.1, 134.9, 139.5, 155.6, 155.7, 170.1, 170.2, 178.3. MS (EI) m/z (%): 583.29 [M]++2 (0.02%), 327.08 (100), 76.99 [C₆H₅]+ (53.09). Elemental analysis for C₃₃H₂₇N₄O₅S₂ (581.71). (Calculated/Found): C, 64.01/64.32; H, 4.68/4.94; N, 12.04/11.75; S 11.02/10.82.

Synthesis of N-(2-(1-(2-cyanoacetyl)-5-methoxy-1H-indol-3-yl)ethyl)acetamide (17)
To a mixture of Melatonin (0.93 g, 4 mmol), ethyl cyanocetate (0.45 g, 4 mmol) in dimethylformamide (20 mL) was added. The reaction mixture was heated under reflux for 5 hrs until all starting materials had disappeared as indicated by TLC. Then left the reaction mixture to cool at room temperature and poured onto ice. The oily layer formed extracted by chloroform (5.20 mL), dried over anhydrous sodium sulphate and filtered off then evaporated. The formed solid product was collected and crystallized from absolute ethanol. Yellow crystals, yield (0.5 g, 41.8%), mp (105-108 °C). IR (KBr, cm⁻¹): ν 3275 (NH), 2989-2827 (CH₃, CH₂), 3078 (CH aromatic), 2214 (CN), 1620, 1585 (2 ≈ C=O), 1554, 1489 (C=C). ¹H NMR (DMSO-d₆, ppm): δ: 1.84 (s, 3H, COCH₃), 2.76 (t, J= 9.60 Hz, 2H, CH₂), 2.36 (s, 2H, CH₂), 3.33 (t, J= 9.20 Hz, 2H, CH₂), 3.75 (s, 3H, OCH₃), 6.69-7.91 (m, J= 8.00 Hz, 4H, C₆H₅, pyrrole-H), 10.60 (s, 1H, NH). MS (EI) m/z (%): 301.25 [M]++2 (0.02%), 299.20 [M]+ (0.02%), 173.15 (100). Elemental analysis for C₁₉H₁₈N₂O₃ (299.32), (% Calculated/Found): C, 64.20/64.55; H, 5.72/5.92; N, 14.04/13.80.

Synthesis of (E)-N-(2-(1-(2-cyanoacetyl)-5-methoxy-1H-indol-3-yl)ethyl)acetamide (18)
To a mixture of compound 17 (1.20 g, 4 mmol) in1,4-dioxane (20 mL) containing piperidine (1.0 mL) benzaldehyde (0.43 g, 4 mmol) was added. The reaction mixture was heated under reflux for 5 hrs until all starting materials had disappeared as indicated by TLC, then left to cool at room temperature, poured onto ice, extracted by chloroform (5.20 mL), dried over anhydrous sodium sulphate and filtered off then evaporated. The formed solid product was collected and crystallized from absolute ethanol. Off white crystals, yield (0.5 g, 85.87%), mp 65-68 °C. IR (KBr, cm⁻¹): ν 3302 (NH), 2986-2827 (CH₃, CH₂), 2100 (CN), 1680, 1631 (2 ≈ C=O), 1585, 1489 (C=C). ¹H NMR (DMSO-d₆, ppm): δ: 1.95 (s, 3H, COCH₃) 2.97 (t, J= 6.68 Hz, 2H, CH₂), 3.61 (t, J= 6.44 Hz,2H, CH₂), 3.87 (s, 3H, OCH₃), 6.88-8.07 (m, J= 8.48 Hz, 9H, C₆H₅, C₆H₃, pyrrole-H), 9.44 (s, 1H, CH), 10.05 (s, 1H, NH). Elemental analysis for C₂₉H₂₃N₂O₅ (387.43), (% Calculated/Found): C, 71.30/71.60; H, 5.46/5.60; N, 10.85/10.66.

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Synthesis of **N-(2-(1-(3-amino-5-phenyl-4,5-dihydro-1H-pyrazole-4-carbonyl)-5-methoxy-1H-indol-3-yl)(ethyl)acetamide** (19)

To a mixture of compound 18 (1.55 g, 4 mmol) in 1,4-dioxiane (20 mL) hydrazine hydrate (0.19 g, 4 mmol) was added. The reaction mixture was heated under reflux for 6 hrs until all starting materials had disappeared as indicated by TLC, then left to cool at room temperature, poured onto ice, extracted by chloroform (5.20 mL), dried over anhydrous sodium sulphate and filtered off then evaporated. The formed solid product was collected and crystallized from absolute ethanol. Off white crystals, yield (0.35 g, 81.85%), mp (102-105 °C). IR (KBr, cm⁻¹): ν 3302 (NH₂), 2989-2827 (CH₃, CH₂), 1631, 1586 (2 C=O), 1554, 1480 (C=C). ¹H NMR (DMSO-d₆, ppm) δ: 1.81 (s, 3H, COCH₃), 2.87 (t, J= 9.60 Hz, 2H, CH₂), 3.30 (t, J= 9.20 Hz, 2H, CH₂), 3.76 (s, 3H, OCH₃), 6.69-7.24 (m, J= 8.80 Hz, 12H, C₆H₅, C₆H₄, pyrrole-H, pyrazoline-CH), 7.92 (s, 2H, NH₂, D₂O exchangeable), 10.63 (s, 1H, NH, D₂O exchangeable). ¹³C NMR (DMSO-d₆, ppm) δ: 23.2, 25.4, 40.60, 44.3, 55.8 (2), 100.6, 111.5, 112.2, 112.5, 123.7 (3), 128.0 (2), 131.9 (2), 153.4 (2), 169.5. Elemental analysis calculated for C₂₃H₂₃N₂O₅ (419.48), (% Calculated/Found): C, 65.85/65.98; H, 6.01/6.30; N, 16.70/16.45.

**Synthesis of ethyl 5-(3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl)-2,4-diaminothiophene-3-carboxylate** (20)

To a mixture of compound 7 (1.09 g, 4 mmol) and ethyl cyanoacetate (0.45 g, 4 mmol) in 1,4-dioxiane (25 mL) containing a catalytic amount of triethylamine (0.5 mL), elemental sulfur (0.13 g, 4 mmol) was added. The reaction mixture, was heated under reflux for 5 hrs until all starting materials had disappeared as indicated by TLC, then left to cool at room temperature, poured onto ice and neutralized with 0.1 N hydrochloric acid, whereby the resulting solid product was collected by filtration and crystallized from the,1,4-dioxiane. Yellow crystals, yield (0.5 g, 46.51%), mp (124-127 °C). IR (KBr, cm⁻¹): ν 3300, 3250 (2NH₂, NH), 3080 (CH aromatic), 2980, 2820 (CH₃, CH₂), 1650, 1715 (2 C=O), 1550, 1485 (C=C). ¹H NMR (DMSO-d₆, ppm) δ: 1.30 (t, 3H, CH₃), 1.81 (s, 3H, COCH₃), 2.77 (t, J= 8.80 Hz, 2H, CH₂), 3.30 (t, J= 6.80 Hz, 2H, CH₂), 3.76 (s, 3H, OCH₃), 4.20 (q, 2H, CH₂), 6.70, 6.73 (2s, 4H, 2NH₂), 7.01-7.94 (m, J= 8.40 Hz, 4H, C₆H₅, pyrrole-H), 10.63 (s, 1H, NH). Elemental analysis calculated for C₂₃H₂₃N₂O₅S (416.49), (% Calculated/Found): C, 57.68/57.97; H, 5.81/5.92; N, 13.45/13.20; S, 7.70/7.50.

**Synthesis of (5)-ethyl 5-(3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl)-4-amino-2-(aminomethyl)-1H-indol-3-yl)propanamido)thiophene-3-carboxylate** (21)

To a mixture of compound 20 (1.72 g, 4 mmol), tryptophan (0.80 g, 4 mmol) in ethanol (30 mL) was added. The reaction mixture was heated under reflux for 7 hrs until all starting materials had disappeared as indicated by TLC, then left to cool at room temperature, poured onto ice and neutralized with 0.1 N hydrochloric acid, whereby the resulting solid product was collected by filtration and crystallized from the,1,4-dioxiane. Dark red crystals, yield (0.5 g, 81.13%), mp (>290) °C. IR (KBr, cm⁻¹): ν 3278 (2NH₂, 3NH), 2989-2828 (CH₃, CH₂), 1710, 1618 (2 C=O), 1551, 1485 (C=C). ¹H NMR (DMSO-d₆, ppm) δ: 1.23 (t, 3H, CH₃), 1.82 (s, 3H, COCH₃), 2.78 (t, , J= 7.20 Hz, 2H, CH₂), 3.33 (t, , J= 7.20 Hz, 2H, CH₂), 3.38 (s, 2H, CH₂), 3.76 (s, 3H, OCH₃), 3.99 (s, 1H, CH), 4.40 (q, 2H, CH₂), 6.71-6.73 (s, 4H, 2NH₂), 7.03-7.50 (m, 9H, C₆H₅, C₆H₄, pyrrole-H), 7.96 (s, 1H, NH), 8.30 (s, 1H, NH), 10.65 (s, 1H, NH-indole). ¹³C NMR (DMSO-d₆, ppm) δ: 22.7 (2), 25.3 (2), 40.1, 55.4 (2), 100.2 (2), 111.1 (2), 111.8 (2), 112.1 (2), 118.1, 121.1, 123.3 (2), 127.7 (2), 131.5 (2), 132.0, 153.1 (2), 169.3 (2). Elemental analysis calculated for C₁₃H₉N₄O₅S (602.70), (% Calculated/Found): C, 61.78/61.98; H, 5.69/5.88; N, 13.94/13.74; S, 5.32/5.10%.

**Anti-tumor Activity Test**

**Chemicals**

Human tumor carcinoma cell lines breast cancer (MCF7) and colon cancer (HCT116) cell lines used in this study were obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.). The tumor cell lines were maintained at the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. Samples were prepared by dissolving 1:1 Stock solution and stored at -20°C in dimethylsulfoxide (DMSO) at 100 µg/mL. Different concentrations of the drug were used 5, 12.5, 25, 50 µg/mL. All chemicals and reagents used in this study are of highest analytical grade. The following chemicals are obtained from:

**A- Sigma Aldrich Chemical Co., St. Louis, Mo, U.S.A.,** was the source of the following chemicals: Dimethylsulphoxide (DMSO), RPMI-1640 medium, trypan blue, Fetal Bovine Serum, Penicillin/ Streptomycin antibiotic and Trypsin-EDTA.
**Determination of Potential Cytotoxicity of Drug on Human Cancer Cell Line**

**Reagents and Buffers**

1. Glacial acetic acid: 1 % was used for dissolving the unbound SRB dye.
2. Sulphorhodamine-B (SRB): 0.4 % concentration was dissolved in 1 % acetic acid was used as a protein dye.
3. Trichloroacetic acid (TCA): 50 % stock solution was prepared, 10 % solution was used for protein precipitation.
4. Tris base, 10 mM, (pH 10.5) was used for SRB dye solubilization. It was prepared by dissolving 121.1 gm of tris base in 1000 ml distilled water and pH was adjusted by 2 M HCl.

**Procedure**

1. Cells were seeded in 96-well microtiter plates at initial concentration of 3x10^4 cell/well in a 150 μl fresh medium and left for 24 hours to attach to the plates.
2. Different concentrations 0, 5, 12.5, 25, 50 μg/ml of drug were added.
3. For each drug concentration, 3 wells were used. The plates were incubated for 48 hours.
4. The plates were fixed with 50 μl cold trichloroacetic acid 10% final concentration for 1 hour at 4 °C.
5. The plates were washed with distilled water using (automatic washer Tecan, Germany) and stained with 50 μl 0.4 % SRB dissolved in 1 % acetic acid for 30 minutes at room temperature.
6. The plates were washed with 1 % acetic acid and air-dried.
7. The dye was solubilized with 100 μl/well of 10M tris base (pH 10.5) and optical density (O.D.) of each well was measured spectrophotometrically at 570 nm with an ELISA microplate reader (Sunrise Tecan reader, Germany). The mean background absorbance was automatically subtracted and means values of each drug concentration was calculated. The experiment was repeated 3 times.

**Calculation**

The percentage of cell survival was calculated as follows:

Surviving fraction = O.D. (treated cells)/ O.D. (control cells).

**Results and Discussion**

**Chemistry**

The target compounds were synthesized through the route as shown in schemes from 1-5. When Melatonin was allowed to react with ethyl chloroacetate in the presence of potassium hydroxide as a catalyst in dry dimethylformamide the ethyl 2-(3-(2-acetamidoethyl)-5-methoxy-1H-indol-1-yl)acetate 2 was afforded after refluxed for 8 hours, compound 2 reacted with hydrazine hydrate in absolute ethanol to give compound 3 (Scheme 1). The IR spectrum of compound 3 shows the presence of NH₃ and 2 C=O groups at 3305 and 1725, 1627, respectively. The ¹H NMR spectrum exhibited the signals of the NH₃ and NH protons. Compound 3 react with phenyl isothiocyanate under reflux in absolute ethanol to afford N-phenyl hydrazinecarbothioamide derivative 4. The IR spectrum of compound 4 showed the presence of NH and C=S groups at 3206 and 1337, 1242, respectively. The ¹H NMR spectrum exhibited the signals of the NH protons. Compound 4 in the presence of ethanolic sodium hydroxide gave the triazole derivative 5 and thia diazole derivative 6 in the presence of concentrated H₂SO₄. The structures of compounds 5 and 6 were confirmed by means of their IR, ¹H NMR, ¹³C NMR, MS and analytical data. On the other hand, Melatonin was reacted with chloroacetonitrile in the presence of anhydrous potassium carbonate in dimethylformamide under stirring to afford compound 7 (Scheme 2), the IR spectrum of compound 7 shows the presence of CN group at ν 2198 cm⁻¹.

Moreover, compound 7 reacted with sodium azide in the presence of ammonium chloride in DMF, and tetrazole derivative 8 was afforded, the IR spectrum of compound 8 showed the disappearance of CN...
group. Compound 8 reacted with ethyl chloroacetate in the presence of anhydrous potassium carbonate in dry acetone under reflux to afford ethyl 2-(2H-tetrazol-2-yl)acetate derivative 9. Also, compound 9 revealed molecular ion peak [M⁺] at m/z 400.75, corresponding to the molecular formula C₇H₁₂N₃O₆. Moreover, compound 9 reacted with hydrazine hydrated in absolute ethanol to give 2-(2H-tetrazol-2-yl)acetohydrazide derivative compound 10. Moreover compound 10 reacted with benzaldehyde in the presence of glacial acetic acid as a catalytic in absolute ethanol under reflux to give compound 11. IR, ¹H NMR, ¹³C NMR and MS data of compound 11 is consistent with the proposed structure. Also Melatonin reacted with malononitrile and sulphur in the presence of thiourea to afford compound 12 [44-46]. (Scheme 3). Compound 12 reacted with phenyl isothiocyanate in the presence of potassium hydroxide in dry dimethylformamide, the obtained intermediate (A) was reacted with appropriate amount of ethyl chloroacetate or phenacyl bromide to give compounds 13 and 14, respectively. The resultant spectral data of IR for compound 13, showed the appearance of the carbonyl group of ester at ν 1720 cm⁻¹ which elucidated the structure. In the ¹H NMR, the presence of the CH₃ group at δ 1.23 ppm and CH₂ group at δ 4.20 ppm confirmed the structure of compound 13. In addition, the structure of compound 14 was confirmed by the existent of C=O at ν 1670 cm⁻¹ in the IR spectrum. Also, the presence of the two phenyl moieties in the ¹H NMR at δ in range 6.69-7.23 ppm, elucidated the structure.

When compounds 13 and 14 were treated with piperidine in absolute ethanol they gave the corresponding thiazole 15 and thiophene derivatives 16, respectively. IR, ¹H NMR, ¹³C NMR and MS data of compound 15 and 16 were consistent with the proposed structure. In the IR spectrum, the presence of the carbonyl group at ν 1630 cm⁻¹ and the absence of the carbonyl group for the ester of compound 15 confirmed its structures. Moreover, compound 15 showed a molecular ion peak [M⁺] at m/z 504.05, corresponding to the molecular formula C₂₀H₂₄N₆O₆S₂ (503.60). There are many other fragments which confirmed the structure of compound 15, such as, [M⁺-2] at m/z 502.09, [M⁺-1] at m/z 502.76, [M⁺+1] at m/z 504.86 and [M⁺+2] at m/z 505.71 and [C₆H₅]⁺ at m/z 77.25.

The structure of the compound 16 was confirmed by the IR spectrum, when the appearance of the amino group (broad peak) at ν 3397 cm⁻¹, and the disappearance of the cyano group. Also, the existence of the amino group in the ¹H NMR spectrum at δ 7.03 ppm elucidated the structure of compound 16. The data of the mass for the latter compound were consistent with the proposed structure and revealed [M⁺+2] at m/z 583.29 which, corresponding to the molecular formula C₁₇H₂₇N₅O₄S₂.

![Scheme 1 Synthesis of Melatonin derivatives 2, 3 and 4; triazole 5 and thiadiazole 6 derivatives](image)

On the other hand, Melatonin reacted with ethyl cyanoacetate in dimethylformamide to give compound 17 (Scheme 4). Moreover, compound 17 reacted with benzaldehyde in 1,4-dioxane containing piperidine to afford N-(2-(1-(2-cyano-3-phenylacryloyl)-5-methoxy-1H-indol-3-yl)ethyl)acetamide 18. The latter compound reacted with hydrazine hydrated, where the addition occurred on the cyano group followed by beta attack and cyclization to afford compound 19. The formation of the amino group in compound 19 was confirmed by D₂O exchangeable ¹H NMR spectrum which revealed characteristic signals at δ= 7.92 ppm and NH group at δ= 10.63 ppm. Also compound 7 reacted with ethyl cyanoacetate and sulphur in the presence of triethylamine and 1,4-dioxane and afforded compound 20 (Scheme 5). On the other hand, compound 20 reacted with tryptophan under reflux 8 hrs in ethanol and gave compound 21. ¹H NMR, IR and Mass data were consistent with the proposed structure.
Scheme 2 Synthesis of Melatonin derivative 7 and tetrazole derivatives 8, 9, 10 and 11

Scheme 3 Synthesis of thiadiazole derivatives 12, 13 and 14; thiazole 15 and thiophene 16 derivatives

Scheme 4 Synthesis of Melatonin derivatives 17, 18 and pyrazole derivative 19

Scheme 5 Synthesis of indolyl thiophene-3-carboxylate derivatives 20 and 21
**In Vitro Cytotoxic Effect**

The cytotoxicity was carried out using Sulphorhodamine-B (SRB) assay following the method reported [54] by SRB was a bright pink aminoxanthrene dye with two sulphonic groups. It was a protein stain that binded to the amino groups of intracellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content. The two cancer cell lines, such as human breast cancer (MCF7) and colon cancer (HCT116) cell lines were used in the evaluation of the newly synthesized compounds as cytotoxic agents (Table 1). Doxorubicin was used as a positive control [55]. Generally, the variations of substituents within the indole ring attached had a notable influence on the cytotoxicity. Figures 1 and 2 described the cytotoxicity of some selected new synthesized compounds against the tested cell lines (MCF7 and HCT116).

**Table 1 In vitro inhibitory effects IC₅₀ (µg/ml) of the newly synthesized compounds against the two human tumor cell lines**

<table>
<thead>
<tr>
<th>Compound Number</th>
<th>Breast cancer (MCF7)</th>
<th>Colon cancer (HCT116)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>11.5</td>
<td>&gt;50</td>
</tr>
<tr>
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<tr>
<td>Doxorubicin</td>
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</tbody>
</table>

¹Drug concentration required to inhibit tumor cell proliferation by 50% after continuous exposure of 48 h; ²Doxorubicin was used as a positive control.

**Structure Activity Relationship**

The reactivity of some selected newly synthesized compounds against the two human cancer cell lines used was summarized in Table 1. The data showed that the compounds 5, 11, 15, and 16 were found to be the most active among the tested compounds against the breast cancer cell lines. Compound 5 showed the most potent effect through all the tested compounds, which due to the presence of the triazole ring with phenyl and SH moieties. Moreover, compounds 15 and 16 revealed high effect due to the existence of thiazolidine and thiophene beside the thiadiazole rings, respectively. Finally, compound 11 showed moderate activity due to the tetrazole ring and a phenyl group. While for the other cell line [colon cancer (HCT116)], the reactivity was reduced and the selected compounds revealed moderate activity. Compounds 6 and 16 revealed a high potent effect compared with the other tested compounds. The activity of the latter compounds was referred to the existence of the thiadiazole and thiophene rings. In conclusion, the presence of the sulfur ring systems enhanced the activity of the compounds towards the different cancer cell lines used, but this conclusion will need several studies to confirm it.
Novel Melatonin Derivatives: Synthesis, Anticancer Evaluations and Molecular-Docking Study

Fig. 1 The cytotoxicity of some newly synthesized compounds against the human breast cancer (MCF7) cell line

Fig. 2 The cytotoxicity of some newly synthesized compounds against the human colon cancer (HCT116) cell line

Molecular Docking Study

To take one step further to determine the mode of action of the tested compounds, molecular-docking study was employed to determine the binding modes against CDK2 (Table 2), and MDM2-p53 protein complex that implicated significantly in cancer disease (Table 3). This target was selected based on its potential roles in apoptosis regulation and limiting cancer progression, therefore, targeting of these macromolecules provides potential benefits in cancer therapy. The cocrystal ligands DTQ for CDK2, and 13Q for P53-MDM2 complex were redocked to assure the validity of the docking parameters and methods to represent the position and orientation of the ligands detected in the crystal structure. The difference of RMSD value between cocrystal ligands to the original cocrystal ligand was <2 Å which approved the accuracy of the docking protocols and parameters [56-58]. Figures 3 and 4 described the interaction of the most promising compound (16) with CDK2 and P53-MDM2 proteins compared to reference ligand (RL).

The in silico modeling studies showed that compounds 6, 11, and 16 were the most effective compounds to bind with the CDK2 binding pocket protein, as they displayed the lowest energy of free compared (-8.7, -9.1, and -10.7 Kcal/mol, respectively) to co-crystallized ligand (-8.3 Kcal/mol). Moreover, compounds 6, 11, and 16 displayed critical hydrogen bond formation, and hydrophobic interaction compared with CDK2 receptor, as compound 6 could form 2 hydrogen bond (LYS89: 3.243 Å, LEU83:3.133 Å), and hydrophobic interaction with 10 amino acid residues (ILE10, VAL18, VAL30, VAL64, LEU148, LEU83, LEU134, LEU298, LEU148, and LEU32) representing by blue colour (Table 2, and Figure 3). Compound 11 formed 2 hydrogen bond (LEUS83: 2.925 Å, GLN131:3.138 Å), and hydrophobic interaction with 11 amino acid residues (ILE10, VAL18, VAL64, VAL163, VAL164, LEU148, LEU83, LEU134, LEU298, LEU148, and LEU32). While compound 16 formed four hydrogen bond (GLU12: 3.150, GLU12:2.440, THR14:3.138, and LYS129:3.259), and hydrophobic interaction with nine amino acid residues (ILE10, VAL18, VAL64, LEU148, LEU83, LEU134, LEU298, LEU148, and LEU134) (Table 2, and Figure 3).

In addition, docking results showed that both compounds 6, and 16 displayed the most promising compounds that can inhibited the MDM2-P53 complex, as they displayed lowest energy of free binding (-8.2, and -8.3 Kcal/mol, respectively), compared to reference ligand (-8.2 Kcal/mol). Moreover, both compound displayed critical hydrogen bond formation, and hydrophobic interaction with the target receptor, as compound 6 formed one hydrogen bond (GLY58: 2.437 Å), and hydrophobic interaction with nine amino acid residues (VAL14, VAL93, LEU54, LEU57, LEU82, ILE19, ILE61, ILE99, and ILE103), while compound 16 formed one hydrogen bond (HIS96: 3.047 Å), and hydrophobic interaction with nine amino acid residues...
According to the aforementioned data, both in vitro cytotoxic data are in agreement with the molecular docking results, revealed that compound 16 are the most potential anti-cancer drugs against both MCF7, and HCT116 cell lines, with proposed potential activity against CDK2, and MDM2-P53 proteins.

Table 2 Results of the docking study of the tested compounds against CDK2 binding pocket in comparison to the co-crystallized ligand

<table>
<thead>
<tr>
<th>Comp. No</th>
<th>Energy of free binding (\Delta G_b)</th>
<th>H-Bond No.</th>
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<th>Length Å</th>
<th>Hydrophobic interaction</th>
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### Table 3 Results of the docking study of the tested compounds against MDM2-p53 binding pocket in comparison to the co-crystallized ligand

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<th>Comp. No</th>
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<th>H-Bond No.</th>
<th>Amino acid</th>
<th>Hydrophobic interaction</th>
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**Fig. 3** The interaction of the most promising compound (16) with CDK2 protein compared to reference ligand (RL). A) 3D interaction, B) hydrogen bond formation, and C) hydrophobic interaction representation by blue color.
Conclusion

This study described the reaction of Melatonin with different chemical reagents to obtain the novel bioactive five-membered heterocyclic compounds containing nitrogen and/or sulfur as a ring system. Some of the newly synthesized products were evaluated as anticancer agents towards two cancer cell lines such as breast cancer (MCF7) and colon cancer (HCT116) cell lines, which revealed that compound 16 was the most active compound towards the two cancer cell lines used. Such results will encourage continued work within the field of Melatonin synthesis. The molecular docking study was carried out for the most promising compound (16).

Conflict of Interest: The authors confirm that there is no conflict of interest.

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Compliance with Ethical Standards

Fig. 4 The interaction of the most promising compound (16) with P53-MDM2 protein compared to reference ligand (RL). A) 3D interaction, B) hydrogen bond formation, and C) hydrophobic interaction representation by blue color

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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