Egypt. J. Chem. **Vol. 64,** No. 3 pp. 1517 - 1533 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

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

Mahmoud Alsayed,[a] Dina S. El-Kady,[b] Mohamed A. Tantawy,[b] Mervat M. AbdElhalim,[b] Samia R. Elazabawy,[a] Amira E. M. Abdallah,[a,*] Gamal A. Elmegeed[b]

[a]*Department of Chemistry, Faculty of Science, Helwan University; Ain Helwan, Cairo 11795, A. R. Egypt*

[b]*Hormones Department, Medical Research Division, National Research Centre, Dokki, Giza, Egypt*

†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 IC_{50} 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

*Corresponding author e-mail: amiraelsayed135@yahoo.com

Receive Date: 13 November 2020, Revise Date: 09 December 2020, Accept Date: 20 December 2020
DOI: 10.21608/EJCHEM.2020.49430.3016

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 ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker at 400 and 100 MHz, respectively, in DMSO-d_6 and dry acetone 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 spectra 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-1*H*-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}): υ 3300 (NH), 3050 (CH-aromatic), 2980, 2891 (CH_3, CH_2), 1720, 1655 (2 C=O), 1580, 1451 (C=C). ^{1}H NMR (Dry acetone, ppm) δ: 1.30 (t, *J*= 7.48 Hz, 3H, CH_3), 1.88 (s, 3H, $COCH_3$), 2.89 (t, *J*= 7.20 Hz, 2H, CH_2), 3.46 (t, *J*= 6.76 Hz, 2H, CH_2), 3.82 (s, 3H, OCH_3), 4.14 (q, *J*= 7.48 Hz, 2H, CH_2), 4.18 (s, 2H, CH_2), 6.74-7.28 (m, *J*= 8.72 Hz, 5H, C_6H_3, NH, pyrrole-H). Elemental analysis for $C_{17}H_{22}N_2O_4$ (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-1*H*-indol-3-yl)methyl)acetamide (3)

A mixture of compound **2** (1.27 g, 4 mmol) and hydrazine hydrated (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 (130-133) °C. IR (KBr, cm^{-1}): υ 3305 (NH_2), 3078 (CH-aromatic), 2989, 2893 (CH_3, CH_2), 1725, 1627 (2 C=O), 1585, 1455 (C=C). ^{1}HNMR (Dry Acetone, ppm) δ: 1.90 (s, 3H, $COCH_3$), 2.74 (t, *J*= 7.24 Hz, 2H, CH_2), 2.90 (s, 2H, CH_2), 3.30 (t, *J*= 7.40 Hz, 2H, CH_2), 3.66 (s, 3H, OCH_3), 6.59-7.13 (m, *J*= 8.76 Hz, 8H, C_6H_3; pyroole-H; 2NH; NH_2). Elemental analysis for $C_{15}H_{20}N_4O_3$ (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-(2-(phenylcarbamothioyl)hydrazinyl)ethyl)-1*H*-indol-3-yl)ethyl)acetamide (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}): υ 3206 (NH), 3113 (NH), 2935, 2854 (CH_3, CH_2), 1685, 1680 (2 C=O), 1597, 1454 (C=C), 1337, 1242 (C=S). ^{1}H NMR (Dry Acetone, ppm) δ: 1.88 (s, 3H, $COCH_3$), 3.00 (m, 4H, $2CH_2$), 3.62 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 7.12-.7.69 (m, *J*= 7.84 Hz, 13H, C_6H_5, C_6H_3; 4NH; pyrrole-H). Elemental analysis $C_{22}H_{25}N_5O_3S$ (439.53), (% Calculated/Found): C, 60.12/60.30; H 5.73/5.90; N 15.93/15.70; S, 7.30/7.01.

Synthesis of *N*-(2-(1-((5-mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)methyl)-5-methoxy-1*H*-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 (205-208) °C. IR (KBr, cm^{-1}): *υ* 3217 (NH), 2927, 2854 (CH_3, CH_2), 1600 (C=O), 1545, 1445 (C=C). ^{1}H NMR (Dry acetone, ppm) *δ*: 1.90 (s, 3H, $COCH_3$), 2.69 (m, 4H, $2CH_2$), 3.50 (s, 3H, OCH_3), 6.82-6.85 (s, 3H, CH_2, pyrrole-H), 7.05-7.55 (m, *J*= 7.80 Hz, 8H, C_6H_5, C_6H_3), 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 $[C_6H_5]^+$ (61.71). Elemental analysis for $C_{22}H_{23}N_5O_2S$ (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)-1*H*-indol-3-yl)ethyl)acetamide (6)

A solution of compound **4** (1.79 g, 4 mmol) was added gradually with stirring to cold conc. H_2SO_4 (10 mL) during 10 min. The mixture was further stirred for another 1 hr in an ice bath. Then the mixture was poured over crushed ice with stirring. The solid separated out was filtered, washed with water, dried and crystallized with ethanol. Off white crystals, yield (0.12 g, 60.64%), mp (151-154) °C. IR (KBr, cm^{-1}): *υ* 3213 (NH), 2981, 2897 (CH_3, CH_2), 1680 (C=O), 1597, 1473 (C=C). ^{1}H NMR (Dry acetone, ppm) *δ*: 1.90 (s, 3H, $COCH_3$), 3.18 (m, 4H, $2CH_2$), 3.60 (s, 3H, OCH_3), 6.89-6.92 (s, 3H, CH_2, pyrrole-H), 7.04-7.55 (m, m, *J*= 7.76 Hz, 8H, C_6H_5, C_6H_3), 9.61 (s, 1H, NH), 12.30 (s, 1H, NH). ^{13}C NMR (DMSO-*d6*, ppm) δ: 39.4 (2), 40.6, 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 $C_{22}H_{23}N_5O_2S$ (421.52), (% Calculated/Found): C, 62.69/62.91; H, 5.50/5.73; N, 16.6116.30/; S, 7.61/7.33.

Synthesis of *N*-(2-(1-(cyanomethyl)-5-methoxy-1*H*-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. chloroacetonitrile (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. Brown crystals, yield (0.95 g, 70.1%), mp (186-189) °C. IR (KBr, cm^{-1}): *υ* 3302 (NH), 2989, 2897 (CH_3, CH_2), 2198 (CN), 1685 (C=O), 1620, 1489 (C=C). ^{1}H NMR (Dry acetone, ppm) *δ*: 1.90 (s, 3H, $COCH_3$), 2.82 (s, 2H, CH_2), 2.91 (t, *J*= 7.28 Hz, 2H, CH_2), 3.49 (t, *J*= 7.44 Hz, 2H, CH_2), 3.81 (s, 3H, OCH_3), 6.75-7.29 (m, *J*= 8.76 Hz, 5H, C_6H_3, pyrrole-H, NH). MS (EI) *m/z* (%): 271.15 [M^+] (0.42), 160.15 (100.00). Elemental analysis for $C_{15}H_{17}N_3O_2$ (271.31), (% Calculated/Found): C, 66.40/66.60; H, 6.32/6.12; N, 15.49/15.62.

Synthesis of *N*-(2-(1-((2H-tetrazol-5-yl)methyl)-5-methoxy-1*H*-indol-3-yl)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 reflex 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}): *υ* 3302 (NH), 2989, 2897 (CH_3, CH_2), 1680 (C=O), 1620, 1489 (C=C), 1550 (C=N). ^{1}H NMR (Dry acetone, ppm) *δ*: 1.89 (s, 3H, $COCH_3$), 2.90 (t, *J*= 7.32 Hz, 2H, CH_2), 3.05 (s, 2H, CH_2), 3.48 (t, *J*= 7.52 Hz, 2H, CH_2),3.82 (s, 3H, OCH_3), 6.74-7.29 (m, *J*= 8.76 Hz, 5H, C_6H_3, pyrrole-H, NH), 9.96 (s, 1H, NH). ^{13}C 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), 57.05 (100.00). Elemental analysis for $C_{15}H_{18}N_6O_2$ (314.34), (% Calculated/Found): C, 57.31/57.60; H, 5.77/5.98; N, 26.74/26.40.

Synthesis of ethyl 2-(5-((3-(2-acetamidoethyl)-5-methoxy-1*H*-indol-1-yl)methyl)-2*H*-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 anhydrous 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}): *υ* 3302 (NH), 2989, 2897 (CH_3, CH_2), 1720, 1680 (2 C=O),

1620, 1480 (C=C), 1550 (C=N). ^{1}H NMR (Dry acetone, ppm) δ: 1.13 (t, J= 8.00 Hz, 3H, CH_3), 1.89 (s, 3H, $COCH_3$), 2.75 (t, J= 7.24 Hz, 2H, CH_2), 3.34 (t, 2H, CH_2), 3.36 (s, 4H, $2CH_2$), 3.66 (s, 3H, OCH_3), 4.13 (q, J= 8.00 Hz, 2H, CH_2), 6.59-7.12 (m, J= 8.72 Hz,, 4H, C_6H_3, pyrrole-H), 9.76 (s, 1H, NH). MS (EI) *m/z* (%): 400.75 [M^+] (0.85%), 160.04 (100.00). Elemental analysis for $C_{19}H_{24}N_6O_4$ (400.43), (% Calculated/Found): C, 56.99/57.20; H, 6.04/6.34; N, 20.99/20.65.

Synthesis of *N*-(2-(1-((2-(2-hydrazinyl-2-oxoethyl)-2*H*-tetrazol-5-yl)methyl)-5-methoxy-1*H*-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 formed 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}): υ 3302 (NH), 2989, 2897 (CH_3, CH_2), 1685, 1680 (2 C=O), 1627, 1489 (C=C), 1550 (C=N). ^{1}H NMR (Dry acetone, ppm) δ: 1.89 (s, 3H, $COCH_3$), 2.75 (t, J= 7.28 Hz, 2H, CH_2), 3.34 (t, J= 7.36 Hz 2H, CH_2), 3.36 (s, 2H, CH_2), 3.66 (s, 3H, OCH_3), 6.60-6.62 (s, 3H, CH_2, pyrrole-H), 6.97-7.13 (m, J= 8.72 Hz, 6H, C_6H_3, NH_2, 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 $C_{17}H_{22}N_8O_3$ (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-(2-benzylidenehydrazinyl)-2-oxoethyl)-2*H*-tetrazol-5-yl)methyl)-5-methoxy-1*H*-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}): υ 3305 (NH), 2927, 2850 (CH_3, CH_2), 1686, 1680 (2 C=O), 1585, 1482 (C=C), 1550 (C=N). ^{1}H NMR (Dry acetone, ppm) δ: 1.88 (s, 3H, $COCH_3$), 2.89 (t, J= 7.28 Hz, 2H, CH_2), 3.02 (s, 3H, CH_3), 3.47 (t, 2H, CH_2), 3.89 (s, 3H, OCH_3), 6.74-6.77 (s, 3H, CH_2, pyrrole-H), 7.05-7.86 (m, J= 8.68 Hz, 9H, C_6H_5, C_6H_3, NH), 8.61 (s, 1H, NH). ^{13}C 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^++1] (0.11%), 161.05 (100.00), 77.08 [C_6H_5]$^+$ (49.27). Elemental analysis for $C_{24}H_{26}N_8O_3$ (474.52), (% Calculated/Found): C, 60.7560.50/; H, 5.52/5.30; N 23.61/23.81.

Synthesis of *N*-(2-(2-(cyanomethyl)-7-methoxy-[1,3,4]thiadiazolo[3,2-*a*]indol-9-yl)ethyl)acetamide (12). 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.

Ethyl 2-((2-(9-(2-acetamidoethyl)-7-methoxy[1,3,4]thiadiazolo[3,2-*a*]indol-2-yl)-2-cyano-1-(phenylamino)vinyl)thio)acetate (13). Brown crystals, yield (1.0 g, 60.7%), mp (144-147) °C. IR (KBr, cm^{-1}): υ 3421, 3244 (2 NH), 3102 (CH aromatic), 2927 (CH_3, CH_2), 2200 (CN), 1720, 1622 (2 C=O), 1564, 1480 (C=C), 1520 (C=N). ^{1}H NMR (DMSO-*d6*, ppm) δ: 1.23 (t, J= 7.48 Hz, 3H, CH_3), 1.82 (s, 3H, $COCH_3$), 2.79 (t, , J= 9.60 Hz, 2H, CH_2), 3.38 (t, , J= 9.20 Hz 2H, CH_2), 3.76 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 4.20 (q, J= 7.48 Hz, 2H, CH_2), 6.88-7.80 (m, , J= 8.80 Hz, 8H, C_6H_5, C_6H_3), 8.12 (s, 1H, NH), 11.24 (s, 1H, NH). Elemental analysis for $C_{27}H_{27}N_5O_4S_2$ (549.66), (% Calculated/Found): C, 59.00/59.33; H, 4.95/5.20; N, 12.74/12.90; S, 11.67/11.30%.

***N*-(2-(2-(1-Cyano-2-((2-oxo-2-phenylethyl)thio)-2-(phenylamino)vinyl)-7-methoxy-[1,3,4]thiadiazolo[3,2-*a*]indol-9-yl)ethyl)acetamide (14)**. Brown crystals, yield (0.8 g, 45.9%), mp (90-93) °C. IR (KBr, cm^{-1}): υ 3304 (NH), 2926 (CH_3, CH_2), 2199 (CN), 1670, 1627 (2 C=O), 1580, 1483 (C=C), 1551 (C=N). ^{1}H NMR (DMSO-*d6*, ppm) δ: 1.80 (s, 3H, $COCH_3$), 2.77 (t, J= 9.20 Hz, 2H, CH_2), 3.31 (t, J= 8.80 Hz, 2H, CH_2), 3.75 (s, 3H, OCH_3), 4.40 (s, 2H, CH_2), 6.69-7.23 (m, J= 8.40 Hz, 13H, 2

C_6H_5, C_6H_3), 7.91, 10.61 (2s, 2H, 2NH). Elemental analysis for $C_{31}H_{27}N_5O_3S_2$ (581.71), (% Calculated/Found): C, 64.01/64.30; H, 4.68/4.92; N, 12.04/11.80; S, 11.02/10.82.

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), piperdine (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}): υ 3431 (NH), 3053 (CH aromatic), 2924, 2854 (CH_3, CH_2), 2194 (CN), 1630, 1590 (2 C=O), 1535, 1488 (C=C). ^{1}H NMR (DMSO-*d6*, ppm) δ: 1.82 (s, 3H, $COCH_3$), 2.80 (t, 2H, CH_2), 3.34 (t, 2H, CH_2), 3.82 (s, 3H, OCH_3), 4.16 (s, 2H, CH_2-thiazolidin), 6.85-7.70 (m, 8H, C_6H_5, C_6H_3), 9.12 (s, 1H, NH). ^{13}C 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), 135.7, 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 $[C_6H_5]^+$ (42.97). Elemental analysis for $C_{25}H_{21}N_5O_3S_2$ (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}): υ broad 3397 (2NH, NH_2), 3057 (CH aromatic), 2921, 2852 (CH_3, CH_2), 1645, 1617 (2 C=O), 1581, 1476 (C=C), 1552 (C=N). ^{1}H NMR (DMSO-*d6*, ppm) δ: 1.82 (s, 3H, $COCH_3$) 2.78 (t, *J*= 8.80 Hz, 2H, CH_2), 3.34 (t, 2H, CH_2), 3.81 (s, 3H, OCH_3), 7.03 (s, 2H, NH_2), 7.14-7.62 (m, *J*= 8.00 Hz, 13H, $2C_6H_5$, C_6H_3), 11.20, 12.20 (2s, 2H, 2NH). ^{13}C 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 $[C_6H_5]^+$ (53.09). Elemental analysis for $C_{31}H_{27}N_5O_3S_2$ (581.71), (% Calculated/Found): C, 64.01/64.32; H, 4.68/4.94; N, 12.04/11.75; S 11.02/10.81.

Synthesis of *N*-(2-(1-(2-cyanoacetyl)-5-methoxy-1*H*-indol-3-yl)ethyl)acetamide (17)

To a mixture of Melatonin (0.93 g, 4 mmol), ethyl cyanoacetate (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}): υ 3275 (NH), 2989-2827 (CH_3, CH_2), 3078 (CH aromatic), 2214 (CN), 1620, 1585 (2 C=O), 1554, 1489 (C=C). ^{1}H NMR (DMSO-*d6*, ppm) δ: 1.84 (s, 3H, $COCH_3$), 2.76 (t, , *J*= 9.60 Hz, 2H, CH_2), 3.26 (s, 2H, CH_2), 3.33 (t, , *J*= 9.20 Hz, 2H, CH_2), 3.75 (s, 3H, OCH_3), 6.69-7.91 (m, , *J*= 8.00 Hz, 4H, C_6H_3, 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 $C_{16}H_{17}N_3O_3$ (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-cyano-3-phenylacryloyl)-5-methoxy-1*H*-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}): υ 3302 (NH), 3082 (CH aromatic), 2986-2827 (CH_3, CH_2), 2100 (CN), 1680, 1631 (2 C=O), 1585, 1489 (C=C). ^{1}H NMR (DMSO-*d6*, ppm) δ: 1.95 (s, 3H, $COCH_3$) 2.97 (t, *J*= 6.68 Hz, 2H, CH_2), 3.61 (t, *J*= 6.44 Hz,2H, CH_2), 3.87 (s, 3H, OCH_3), 6.88-8.07 (m, , *J*= 8.48 Hz, 9H, C_6H_5, C_6H_3, pyrrole-H), 9.44 (s, 1H, CH), 10.05 (s, 1H, NH). Elemental analysis for $C_{23}H_{21}N_3O_3$ (387.43), (% Calculated/Found): C, 71.30/71.60; H, 5.46/5.60; N, 10.85/10.66.

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

To a mixture of compound 18 (1.55 g, 4 mmol) in 1,4-dioxane (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}): υ 3302 (NH_2, 2NH), 3082 (CH), 2989-2827 (CH_3, CH_2), 1631, 1586 (2 C=O), 1554, 1480 (C=C). ^{1}H NMR (DMSO-*d6*, ppm) δ: 1.81 (s, 3H, $COCH_3$), 2.78 (t, *J*= 9.60 Hz, 2H, CH_2), 3.30 (t, *J*= 9.20 Hz, 2H, CH_2), 3.76 (s, 3H, OCH_3), 6.69-7.24 (m, *J*= 8.80 Hz, 12H, C_6H_5, C_6H_3, pyrrole-H, pyrazoline-CH), 7.92 (s, 2H, NH_2, D_2O exchangeable), 10.63 (s, 1H, NH, D_2O exchangeable). ^{13}C 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 $C_{23}H_{25}N_5O_3$ (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-1*H*-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-dioxane (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 the1,4-dioxane. Yellow crystals, yield (0.5 g, 46.51%), mp (124-127) °C. IR (KBr, cm^{-1}): υ 3300, 3250 ($2NH_2$, NH), 3080 (CH aromatic), 2980, 2820 (CH_3, CH_2), 1650, 1715 (2 C=O), 1550, 1485 (C=C). ^{1}H NMR (DMSO-*d6*, ppm) δ: 1.30 (t, 3H, CH_3), 1.81 (s, 3H, $COCH_3$), 2.77 (t, *J*= 8.80 Hz, 2H, CH_2), 3.30 (t, *J*= 6.80 Hz, 2H, CH_2), 3.76 (s, 3H, OCH_3), 4.20 (q, 2H, CH_2), 6.70, 6.73 (2s, 4H, $2NH_2$), 7.01-7.94 (m, *J*= 8.40 Hz, 4H, C_6H_3, pyrrole-H), 10.63 (s, 1H, NH). Elemental analysis for $C_{20}H_{24}N_4O_4S$ (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 (*S*)-ethyl 5-(3-(2-acetamidoethyl)-5-methoxy-1*H*-indol-1-yl)-4-amino-2-(2-amino-3-(1*H*-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 the1,4-dioxane. Dark red crystals, yield (0.5 g, 81.13%), mp (>290) °C. IR (KBr, cm^{-1}): υ 3278 ($2NH_2$, 3NH), 2989-2828 (CH_3, CH_2), 1710, 1618 (2 C=O), 1551, 1485 (C=C). ^{1}H NMR (DMSO-*d6*, ppm) δ: 1.23 (t, 3H, CH_3), 1.82 (s, 3H, $COCH_3$), 2.78 (t, , *J*= 7.20 Hz, 2H, CH_2), 3.33 (t, , *J*= 7.20 Hz, 2H, CH_2), 3.38 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.99 (s, 1H, CH), 4.40 (q, 2H, CH_2), 6.71-6.73 (s, 4H, $2NH_2$), 7.03-7.50 (m, 9H, C_6H_4, C_6H_3, pyrrole-H), 7.96 (s, 1H, NH), 8.30 (s, 1H, NH), 10.65 (s, 1H, NH-indole). ^{13}C 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 for $C_{31}H_{34}N_6O_5S$ (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.

B- Applichem, Germanywas the source of Tris buffer.

Cells and Culture Conditions

Reagents and Buffers

The procedure which applied was carried out by the method reported in the literature [47].

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 $3x10^3$ 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 cells were fixed with 50 µl cold trichloroacetic acid 10% final concentration for 1 hour at 4 ^{0}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).

Molecular Docking Study

The structures of all tested compounds were modelled using the Chemsketch software (http://www.acdlabs.com/resources/freeware/). The structures were optimized and energy minimized using VEGAZZ software [48]. The optimized compounds were used to perform molecular docking against, cyclin dependent Kinase 2, and p53 binding site in MDM2. The three-dimensional structure of the molecular target was obtained from Protein Data Bank (PDB) (www.rcsb.org): (PDB: 1DI8, https://www.rcsb.org/structure/1di8), (PDB: 2LZg, https://www.rcsb.org/structure/2lzg). The steps for receptor preparation included the removal of heteroatoms (water and ions), the addition of polar hydrogen, and the assignment of charge. The active sites were defined using grid boxes of appropriate sizes around the bound co-crystal ligands. The docking study was performed using Autodock vina [49] and Chimera for visualization [50]. All docking procedures and scoring were recorded according to our previous publications [51-53].

Results and Discussion

Chemistry

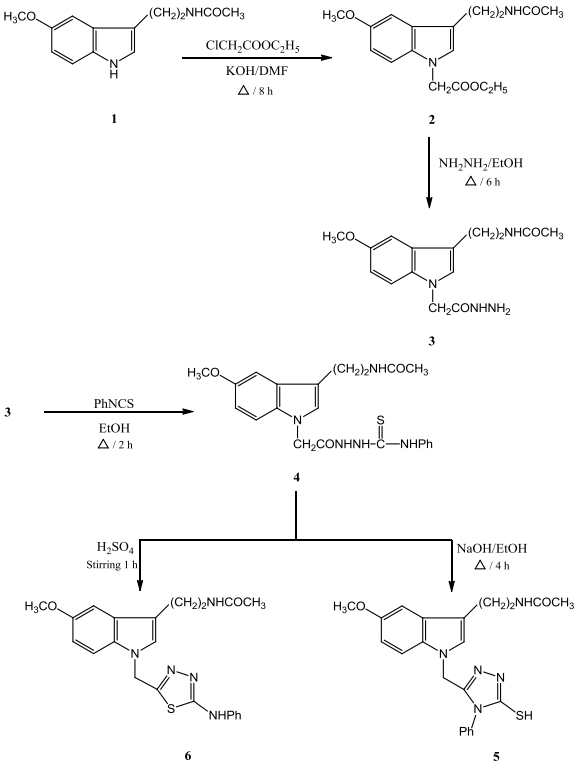
The target compounds were synthesized through the route as shown in schemes from 1-5. When Melatonin was allowed to react with ethyl chloroacetate in the presence of potassium hydroxide as a catalyst in dry dimethylformamide the ethyl 2-(3-(2-acetamidoethyl)-5-methoxy-1*H*-indol-1-yl)acetate **2** was 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 NH_2 and 2 C=O groups at 3305 and 1725, 1627, respectively. The ^{1}H NMR spectrum exhibited the signals of the NH_2 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 ^{1}H NMR spectrum exhibited the signals of the NH protons. Compound **4** in the presence of ethanolic sodium hydroxide gave the triazole derivative **5** and thiadiazole derivative **6** in the presence of concentrated H_2SO_4. The structures of compounds **5** and **6** were confirmed by means of their IR, ^{1}H NMR, ^{13}C 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 υ 2198 cm^{-1}.

Moreover, compound **7** reacted with sodium azide in the presence of ammonium chloride in DMF, 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-(2*H*-tetrazol-2-yl)acetate derivative **9**. Also, compound **9** revealed molecular ion peak [M^+] at *m/z* 400.75, corresponding to the molecular formula $C_{19}H_{24}N_6O_4$. Moreover, compound **9** reacted with hydrazine hydrated in absolute ethanol to give 2-(2*H*-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, ^{1}H NMR, ^{13}C NMR and MS data of compound **11** is consistent with the proposed structure. Also Melatonin reacted with malononitrile and sulphur in the presence of triethylamine to afford compound **12** [44-46], **(Scheme 3)**. Compound **12** reacted with phenyl isothiocynate 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 υ 1720 cm^{-1} which elucidated the structure. In the ^{1}H NMR, the presence of the CH_3 group at δ 1.23 ppm and CH_2 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 υ 1670 cm^{-1} in the IR spectrum. Also, the presence of the two phenyl moieties in the ^{1}H 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, ^{1}H NMR, ^{13}C 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 υ 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 $C_{25}H_{21}N_5O_3S_2$ (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 $[C_6H_5]^+$ at *m/z* 77.25.

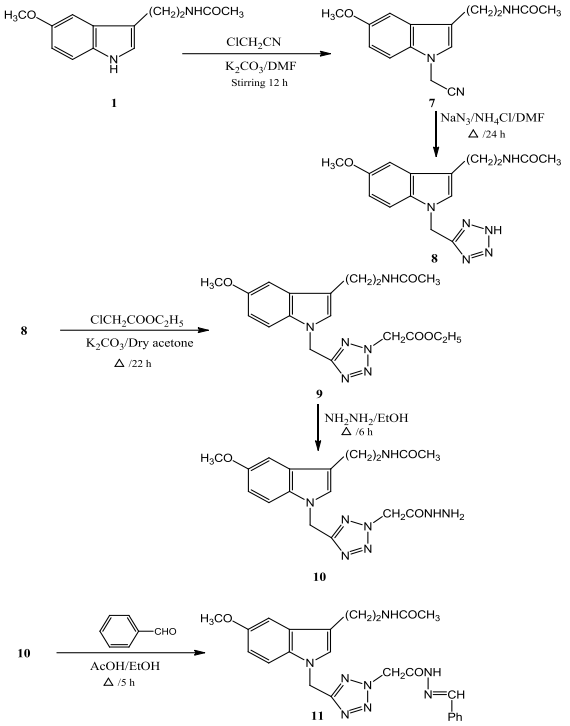
The structure of the compound **16** was confirmed by the IR spectrum, when the appearance of the amino group (broad peak) at υ 3397 cm^{-1}, and the disappearance of the cyano group. Also, the existence of the amino group in the ^{1}H NMR spectrum at δ 7.03 ppm elucidated the structure of compound **16**. 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 $C_{31}H_{27}N_5O_3S_2$.



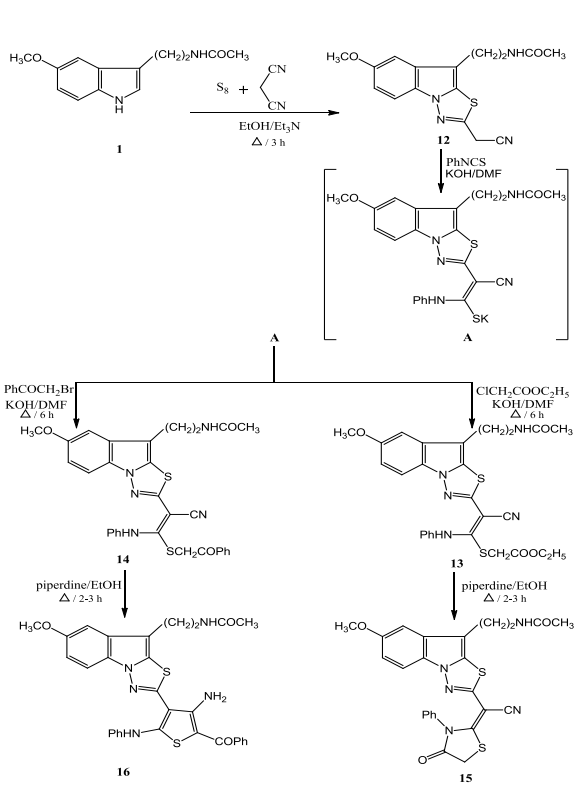
Scheme 1 Synthesis of Melatonin derivatives **2**, **3** and **4**; triazole **5** and thiadiazole **6** derivatives

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-1*H*-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 D_2O exchangeable ^{1}H NMR spectrum which revealed characteristic signals at δ= 7.92 ppm and NH group at δ= 10.63 ppm.

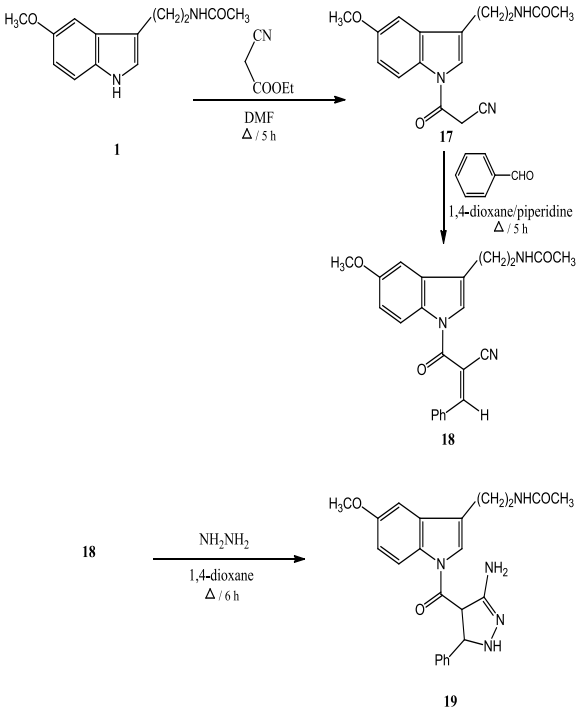
Also compound **7** reacted with ethyl cyanoacetate and sulphur in the presence of triethylamine and 1,4-dioxane and afforded compound **20 (Scheme 5)**. On the other hand, compound **20** reacted with tryptophan under reflux 8 hrs in ethanol and gave compound **21**. ^{1}H NMR, IR and Mass data were consistent with the proposed structure.



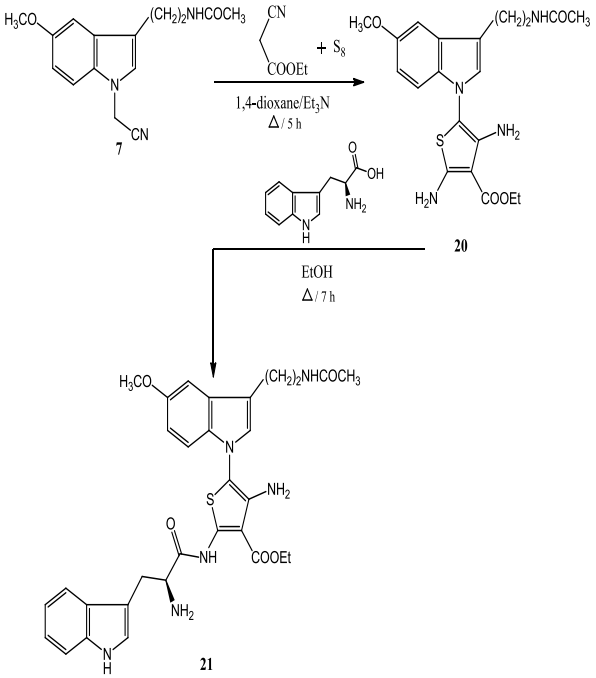
Scheme 2 Synthesis of Melatonin derivative **7** and tetrazole derivatives **8**, **9**, **10** and **11**



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 IC_{50} (µg/ml) of the newly synthesized compounds against the two human tumor cell lines

Compound Number	IC_{50} (µg/ml)[1]	
	Breast cancer (MCF7)	**Colon cancer (HCT116)**
5	11.5	>50
6	21.5	37.5
8	33.5	42.0
11	18.5	43.0
15	17.0	41.0
16	17.0	39.8
19	29.5	48.0
Doxorubicin	3.53	3.73

[1]Drug concentration required to inhibit tumor cell proliferation by 50% after continuous exposure of 48 h;
[*]Doxorubicin 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.

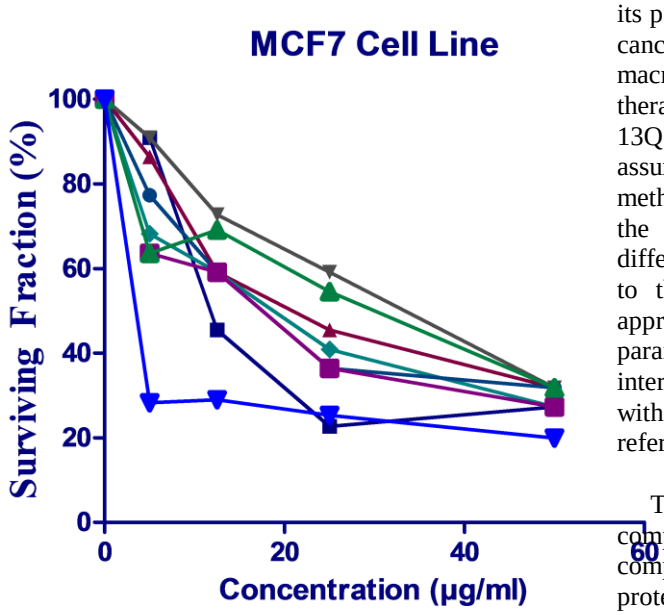


Fig. 1 The cytotoxicity of some newly synthesized compounds against the human breast cancer (MCF7) cell line

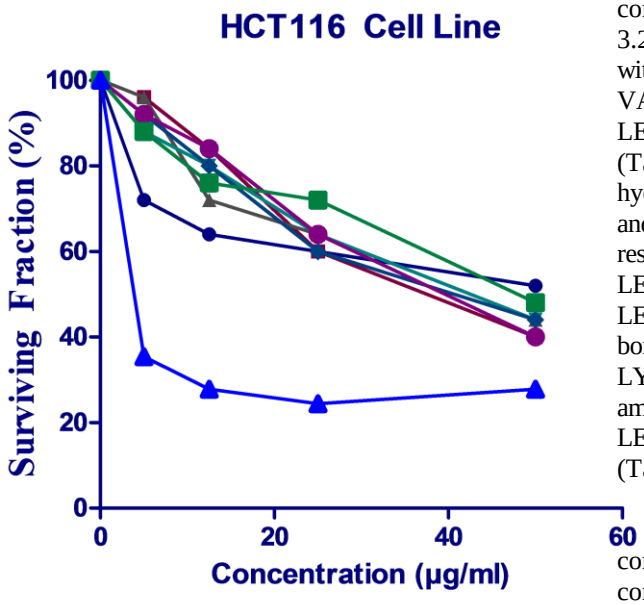


Fig. 2 The cytotoxicity of some newly synthesized compounds against the humancolon cancer (HCT116) cell line

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 ligand detected in the crystal structure. The difference of RMSD value between cocrystal ligands to the original cocrystal ligand was <2Å 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-crystalized 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: 3.243Å, 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: 2.925Å, 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

(VAL14, VAL93, LEU54, LEU57, LEU82, ILE19, ILE61, ILE99, and ILE103), compared to reference ligand that formed one hydrogen bond (HIS96 :2.110), and hydrophobic interaction with 10 amino acid residues (VAL14, VAL93, LEU54, LEU57, ILE61, ILE99, PHE86, PHE91, HIS96, and TYR100).

According to the afromentioned data, both in vitro cytotoxic data are in agreement with the molecular docking results, revealed that compound **16** are the most potentail anti-cancer drugs against both MCF7, and HCT116 cell lines, with proposed potentail 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

Comp. No	*CDK2* Energy of free binding ΔG_b [a]	H-Bond No.	Amino acid	Length Å	Hydrophobic interaction
5	-8.4	3	THR14 THR14 ASN132	2.975 3.159 2.411	ILE10, VAL18, VAL64, LEU148, LEU32, LEU134, LEU148, LEU78
6	-8.7	2	LYS89 LEU83	3.243 3.133	ILE10, VAL18, VAL30, VAL64, LEU148, LEU83, LEU134, LEU298, LEU148, LEU32
8	-8.1	2	GLN131 ASP145	2.320 3.167	ILE10, ILE135, VAL18, VAL64, LEU148, LEU83, LEU134, LEU298, LEU148, LEU32
11	-9.1	2	LEU83 GLN131	2.925 3.458	ILE10, VAL18, VAL64, VAL163, VAL164, LEU148, LEU83, LEU134, LEU298, LEU148, LEU32
15	-7.4	3	ASP86 LYS33 THR14	3.206 3.434 3.100	ILE10, VAL18, VAL64, VAL163, VAL164, LEU148, LEU83, LEU134, LEU298, LEU148, LEU32
16	-10.7	4	GLU12 GLU12 THR14 LYS129	3.190 2.440 3.138 3.259	ILE10, VAL18, VAL64, LEU148, LEU83, LEU134, LEU298, LEU148, LEU134
19	-7.6	1	GLU12	2.860	ILE10, VAL18, VAL64, VAL163, VAL164, LEU83, LEU134, LEU298
Reference ligand (DTQ)	-8.3	2	LYS33 LEU83	2.831 2.803	ILE10, VAL18, VAL64, LEU148, LEU83, LEU133, LEU134, LEU184, PHE82

Table 3 Results of the docking study of the tested compounds against **MDM2-p53** binding pocket in comparison to the co-crystallized ligand

Comp. No	*MDM2-P53* Energy of free binding ΔG_b [a]	H-Bond			Hydrophobic interaction
		No.	Amino acid	Length Å	
5	-7.8	1	HIS96	2.457	VAL14, VAL18, VAL93, LEU54, LEU57, LEU82, ILE19, ILE61, ILE99, ILE103
6	-8.2	1	GLY58	2.487	VAL14, VAL93, LEU54, LEU57, LEU82, ILE19, ILE61, ILE99, ILE103
8	-6.9	-	-	-	VAL14, VAL53, VAL93, LEU54, LEU57, ILE19, ILE61, ILE82, ILE99, ILE103
11	-7.5	1	GLY58	2-109	VAL14, VAL93, LEU54, LEU57, ILE19, ILE61, ILE99, ILE103
15	-7.6	-	-	-	VAL14, VAL93, LEU54, LEU57, LEU82, ILE19, ILE99, ILE103
16	-8.3	1	HIS96	3.047	VAL14, VAL93, LEU54, LEU57, LEU82, ILE19, ILE61, ILE99, ILE103
19	-7.1	-	-	-	VAL14, VAL93, LEU54, LEU57, LEU82, ILE19, ILE61, ILE99, ILE103
Reference ligand (13Q)	-8.2	1	HIS96	2.11	VAL14, VAL93, LEU54, LEU57, ILE61, ILE99, PHE86, PHE91, HIS96, TYR100

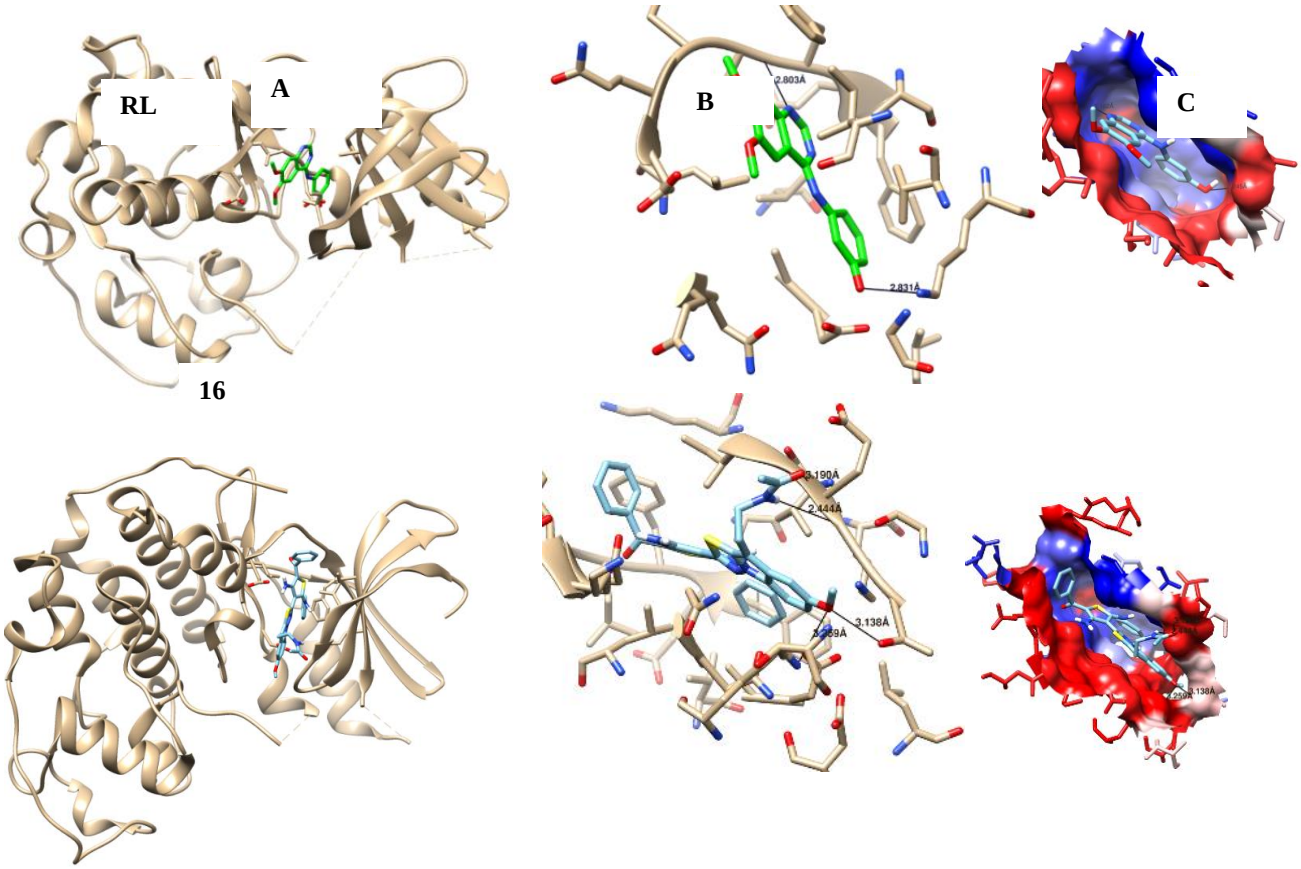


Fig. 3 The interaction of the most promising compound (**16**) with CDK2 protein compared to reference ligand (RL), **A)** 3D interaction, **B)** hydrogen bond formation, and **C)** hydrophobic interaction representation by blue color

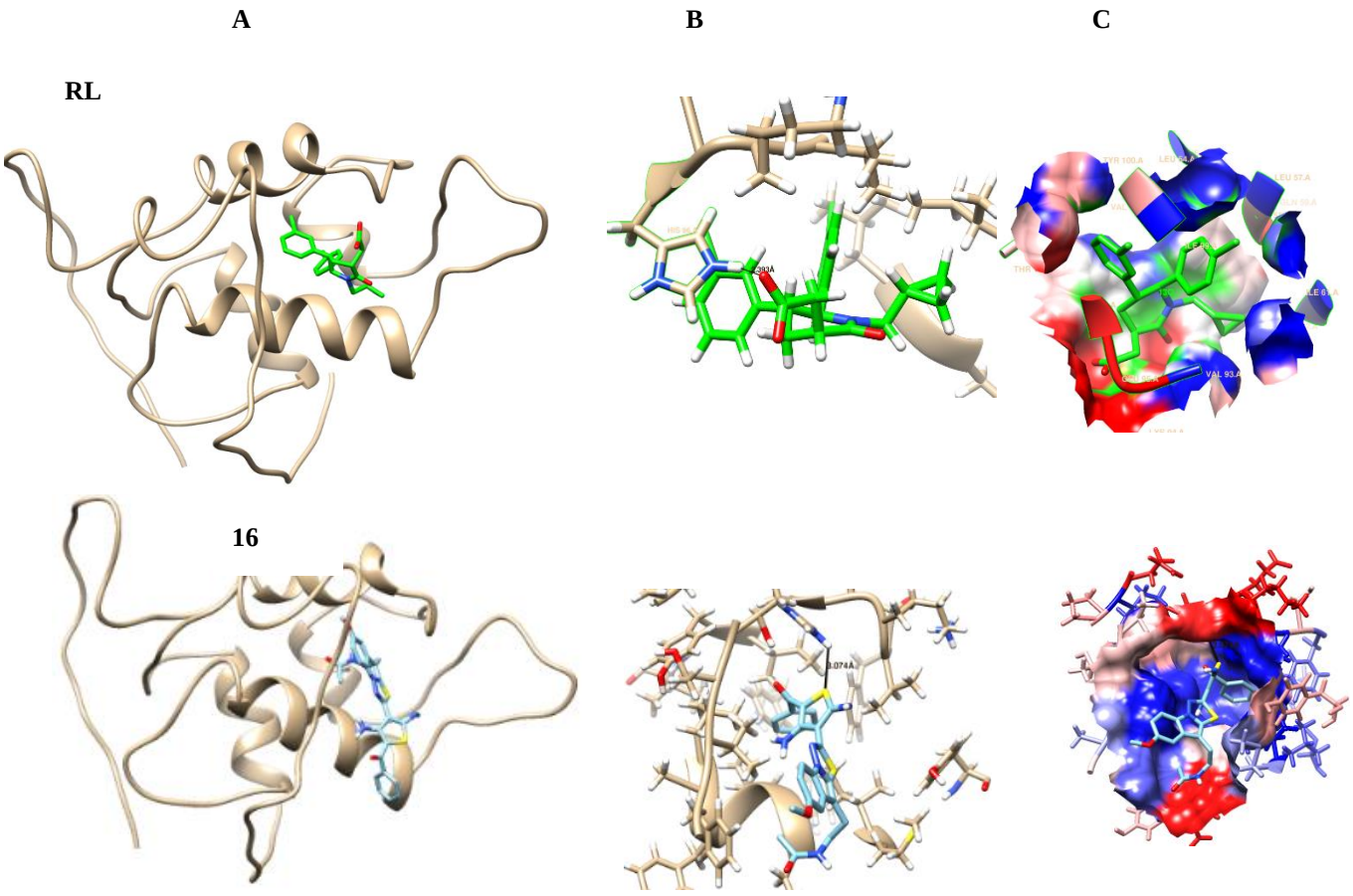


Fig. 4 The interaction of the most promising compound (**16**) with P53-MDM2 protein compared to reference ligand (RL), **A)** 3D interaction, **B)** hydrogen bond formation, and **C)** hydrophobic interaction representation by blue color

Conclusion

This stu the reaction of Melatonin with different gents 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

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

Funding Information The authors acknowledge the financial support of National Research Center, Cairo, Egypt: Grant No. 12010124 for Prof Gamal A. Elmegeed.

Compliance with Ethical Standards

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

References

[1] Wang S. Y., Shi X. C., Indole-based melatonin analogues: Synthetic approaches and biological activity. *European Journal of Medicinal Chemistry*, **185**, 111847 (2019).

[2] Hickman A. B., Klein D. C., Dyda F., Melatonin biosynthesis: the structure of serotonin N-acetyltransferase at 2.5 A resolution suggests a catalytic mechanism. *Molecular Cell*, **3** (1), 23-32 (1999).

[3] Ye T., Yin X., Yu L., Zheng S. J., Cai W. J., Wu Y., Feng Y. Q., Metabolic analysis of the melatonin biosynthesis pathway using chemical labeling coupled with liquid chromatography-mass spectrometry. *Journal Pineal Research*, **66** (1), e12531 (2019).

[4] Back K, Tan D.-X., Russel J Reiter, R. J., Melatonin biosynthesis in plants: multiple pathways catalyze tryptophan to melatonin in the cytoplasm or chloroplasts. *Journal Pineal Research*, **61** (4), 426-437 (2016).

[5] Zhao D., Yu Y., Shen Y., Liu Q., Zhao Z., Sharma R., Reiter R. J., Melatonin Synthesis and Function: Evolutionary History in Animals and Plants. *Frontiers Endocrinology (Lausanne)*. **10**, 249 (2019).

[6] Tan DX, Manchester LC, Liu X, Rosales-Corral SA, Acuna-Castroviejo D, Reiter RJ., Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. *Journal Pineal Research*. **54** (2), 127-138 (2013).

[7] Estevão M. S., Carvalho L. C., Ribeiro D., Couto D., Freitas M., Gomes A., Ferreira L. M., Fernande, E., Marques M. M., Antioxidant activity of unexplored indole derivatives: synthesis and screening. *European Journal of Medicinal Chemistry*. **45** (11), 4869-4878 (2010).

[8] de Zanette S. A., Vercelino R., Laste G., Rozisky J. R., Schwertner A., Machado C. B., Xavier F., de Souza I. C., Deitos A., Torres I. L., Caumo, W., Melatonin analgesia is associated with improvement of the descending endogenous pain-modulating system in fibromyalgia: a phase II, randomized, double-dummy, controlled trial. *BMC Pharmacology Toxicol*ogy. **15**, 40 (2014).

[9] Chen S. J., Huang S. H., Chen J. W., Wang K. C., Yang Y. R., Liu P. F., Lin G. J., Sytwu H. K., Melatonin enhances interleukin-10 expression and suppresses chemotaxis to inhibit inflammation in situ and reduce the severity of experimental autoimmune encephalomyelitis. *International Immunopharmacology*. **31**, 169-177 (2016).

[10] Anderson G., Rodriguez M., Multiple sclerosis: the role of melatonin and *N*-acetylserotonin. *Multiple Sclerosis Related Disorders*, **4** (2), 112-123 (2015).

[11] Lochner A., Marais E., Huisamen B., Melatonin and cardioprotection against ischaemia/reperfusion injury: What's new? A review. *Journal Pineal Research*, **65** (1), e12490 (2018).

[12] Lin H. W., Lee E. J. Effects of melatonin in experimental stroke models in acute, sub-acute, and chronic stages. *Neuropsychiatric Disease Treatment*, **5**, 157-162 (2009).

[13] Rahman A., Hasan A. U., Kobori H., Melatonin in chronic kidney disease: a promising chronotherapy targeting the intrarenal renin-angiotensin system. *Hypertension Research*. **42** (6), 920-923 (2019).

[14] Alonso-Alconada D., Alvarez A., Arteaga O., Martínez-Ibargüen A., Hilario E., Neuroprotective effect of melatonin: a novel therapy against perinatal hypoxia-ischemia. *International Journal of Molecular Science*, **14** (5), 9379-9395 (2013).

[15] Gupta V., A Review on Biological Activity of Imidazole and Thiazole Moieties and their Derivatives, *Science International*, **1** (7), 253-260 (2013).

[16] Di Bella G., Mascia F., Gualano L., Di Bella L., Melatonin anticancer effects: review. *International Journal of Molecular Science*, **14** (2), 2410-2430 (2013).

[17] Morera-Fumero A. L., Abreu-Gonzalez ,P., Role of melatonin in schizophrenia. *International Journal of Molecular Science*, **14** (5), 9037-9050 (2013).

[18] Gagnon K., Godbout R., Melatonin and Comorbidities in Children with Autism Spectrum Disorder. *Current Developmental Disorders Reports*. **5** (3), 197-206 (2018).

[19] Jain S.V., Horn P. S., Simakajornboon N., Beebe D. W., Holland K., Byars A. W., Glauser T. A., Melatonin improves sleep in children with epilepsy: a randomized, double-blind, crossover study. *Sleep Medicine*, **16** (5), 637-644 (2015).

[20] Cardinali D. P., Srinivasan V., Brzezinski A., Brown G. M., Melatonin and its analogs in insomnia and depression. *Journal Pineal Research*, **52** (4), 365-375 (2012).

[21] Braam W., Didden R., Smits M., Curfs L., Melatonin treatment in individuals with intellectual disability and chronic insomnia: a randomized placebo-controlled study. *Journal of Intellectual Disability Research*, **52** (Pt 3), 256-64 (2008).

[22] Richdale A. L., Baker E. K.. Sleep in individuals with an intellectual or developmental disability: Recent research reports. *Current Developmental Disorders Reports*, **1** (2), 74-85 (2014).

[23] Zhang R., Wang X., Ni L., Di X., Ma B., Niu S., Liu C., Reiter R. J. COVID-19: Melatonin as a potential adjuvant treatment. *Life Science*, **250**, 117583 (2020).

[24] Herrera E. A., González-Candia A. Comment on Melatonin as a potential adjuvant treatment for COVID-19. *Life Science*, **253**, 117739 (2020).

[25] Shneider A., Kudriavtsev A., Vakhrusheva A. Can melatonin reduce the severity of COVID-19 pandemic? *International Reviews Immunology*. **39** (4), 153-162 (2020).

[26] Reddyrajula R., Dalimba U., Kumar S.M. Molecular hybridization approach for phenothiazine incorporated 1,2,3-triazole hybrids as promising antimicrobial agents: Design, synthesis, molecular docking and in silico ADME studies. *European Journal of Medicinal Chemistry*, **168**, 263-282 (2019).

[27] Abdallah A. E. M., Elgemeie G. H. Design, Docking, Synthesis and Antimicrobial Evaluation of Some Novel Pyrazolo[1,5-*a*]pyrimidines and their Corresponding Cycloalkane Ring-Fused Derivatives as Purine Analogues. *Drug Design Development Theraoy*, **12**, 1785-1798 (2018).

[28] Elkanzia N. A. A., El-Sofany W. I., Gaballah S. T., Mohamed A. M., Kutkat O., El-Sayed W. A. Synthesis, molecular modeling, and antiviral activity of novel triazole nucleosides and their analogs. *Russin Journal of General Chemistry*, **89**, 1896–1904 (2019).

[29] Phatak P. S., Bakale R. D., Dhumal S. T., Dahiwade L. K., Choudhari P. B., Krishna V. S., Sriram D., Haval K. P. Synthesis, antitubercular evaluation and molecular docking studies of

phthalimide bearing 1,2,3-triazoles. *Synthetic. Communication*. **49**, 2017–2028 (2019).

[30] Leal J. G., Sauer A. C., Mayer J. C. P., Stefanello S. T. Synthesis and electrochemical and antioxidant properties of chalcogenocyanateoxadiazole and 5-heteroarylchalcogenomethyl-1*H*-tetrazole derivatives. *New Journal of Chemistry*, **41** (13), 5875-5883 (2017).

[31] Jakovljević K., Matić I. Z., Stanojković T., Krivokuća A., Marković V., , Joksović M. D. , Mihailović N., Nićiforović M. , Joksović L. Synthesis, antioxidant and antiproliferative activities of 1,3,4-thiadiazoles derived from phenolic acids. *Bioorgganic Medicinal Chemistry Letters*. **27** (16), 3709-3715 (2017).

[32] Ahmadi F., Ghayahbashi M. R., Sharifzadeh M., Alipoiur E., Ostad S. N., Vosooghi M., khademi H. R., Amini M. Synthesis and evaluation of anti-inflammatory and analgesic activities of new 1,2,4-triazole derivatives. *Medicinal Chemistry*, **11** (1), 69-76 (2014).

[33] Molvi K. I., Mansuri M., Sudarsanam V., Patel M. M., Andrabi S. M. A., Haque N. Synthesis, anti-inflammatory, analgesic and antioxidant activities of some tetrasubstituted thiophenes. *Journal of Enzyme Inhibition and Medicinal Chem*istry, **23** (6), 829-838 (2008).

[34] Wardakhan W. W., El-sayed N. N. E. Synthesis and Evaluation of Antidepressant and Sedative Activities of Some Benzo[*B*] thiophenes. *Egyptain. Journal of Chemistry*, **53** (4), 515-526 (2010).

[35] Bailey D. M., Hansen P. E., Hlavac A. G., Baizman E. R. Pearl J., DeFelice A. F., Feigenson M. E. 3,4-Diphenyl-1H-pyrazole-1-propanamine antidepressants. *Journal of Medicinal Chemistry*, **28** (2), 256-260 (1985).

[36] Bhat M., Belagali S. L., Kumar N. K. H., Jagannath S., Anti-mitotic Activity of the Benzothiazole-pyrazole Hybrid Derivatives. *Anti-Infective Agents in Medicinal Chemistry*, **17** (1), 66-73 (2019).

[37] Romagnoli R., Baraldi P. G., Carrion M. D., Cara C. L., Preti D., Fruttarolo F., Pavani M. G.,Tabrizi M. A. Tolomeo M., Grimaudo S., Cristina A. D., Balzarini J., Hadfield J. A., Brancale A., Hamel E. Synthesis and biological evaluation of 2- and 3-aminobenzo[b]thiophene derivatives as antimitotic agents and inhibitors of tubulin polymerization. *Journal of Medicinal Chemistry*, **50** (9), 2273-2277 (2007)

[38] Abdallah A. E. M., Mohareb R. M., Ahmed E. A. Novel Pyrano[2,3-*d*]thiazole and Thiazolo[4,5-*b*]pyridine Derivatives: One-Pot Three-Component Synthesis and Biological Evaluation as Anti-cancer Agents, c-Met and Pim-1 kinase Inhibitors. *Journal of Heterocyclic Chemistry*, **56**, 3017-3029 (2019).

[39] Mohamed S. A., El-Kady D. S., Abd Rabou A. A., Tantawy M. A., AdbElhalim M. M., Elazabawy S. R. . Abdallah A. E. M., Gamal A. Elmegeed. Synthesis of Novel Hybrid Hetero-Steroids: Molecular docking Study Augmented Anti-Proliferative Properties Against Cancerous cells. *Steroids*, **154**, 1082527 (2020).

[40] Mohareb R. M., Abdallah A. E. M., Ahmed E. A. The synthesis and cytotoxicity evaluations of thiazole derivatives derived from 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thio-phene-3-carbonitrile. *Acta Pharm*aceutica, **67** (3), 495–510 (2017).

[41] Mohareb R. M., Khalil E. M., Mayhoub A. E., Abdallah A. E. M. Synthesis and Anti-tumor Evaluation of Novel Pyran, Thiophene and Pyridine Derivatives Incorporating Thiazole Ring. *Journal of Heterocyclic Chemistry*, **57** (3): 1330-1343 (2020).

[42] Kassem A. F., Abbas E. M. H., El-Kady D. S., Awad H. M., El-Sayed W. A. Synthesis, docking studies and anticancer activity of new tetrazolyl-and (triazolyl)thiazole glycosides and acyclic analogs. *Mini-Rev. Medicinal Chemistry*, **19**, 933–948 (2019).

[43] Sayed A. R., Gomha S. M., Taher E. A., Muhammad Z. A., El-Seedi H. R., Gaber H. M., Ahmed M. M. One-Pot Synthesis of Novel Thiazoles as Potential Anti-Cancer Agents. *Drug Design Development Theraoy*. **14**, 1363–1375 (2020).

[44] Doss S. H., Mohareb R. M., Elmegeed G. A., Luoca N. A. Synthesis and study of pigment aggregation response of some melatonin derivatives. *Pharmazie*. **58** (9), 607-613 (2003).

[45] Ahmed H. H., Elmegeed G. A., el-Sayed el-S. M., Abd-Elhalim M. M., Shousha W. G., Shafic R. W., Potent neuroprotective role of novel melatonin derivatives for management of central neuropathy induced by acrylamide in rats. *European Journal of Medicinal Chemistry*, **45** (11), 5452-5459 (2010).

[46] Elmegeed G. A., Khalil W. K., Raouf A. A., Abdelhalim M. M., Synthesis and in vivo anti-mutagenic activity of novel melatonin derivatives. *European Journal of Medicinal Chemistry*. **43** (4), 763-770 (2008).

[47] Skehan P., Storeng R., Scudiero D., Monk,s A., McMahon J., Vistica D., Warren J. T., Bokesch H., Kenney S., Boyd M. R., New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute.*, **82** (13), 1107-1112 (1990).

[48] Pedretti A., Villa L., Vistoli G., VEGA--an open platform to develop chemo-bio-informatics applications, using plug-in architecture and script programming. Journal of Computer Aided Moleculare Design. **18** (3), 167-73 (2004).

[49] Trott O., Olson A. J., AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, **31**(2), 455-61(2010).

[50] Pettersen E. F., Goddard T. D., Huang C. C., Couch G. S., Greenblatt D. M., Meng E. C., Ferrin T. E., UCSF Chimera--a visualization system for exploratory

research and analysis. *Journal of Computational Chemistry*. **25** (13), 1605-1612 (2004).

[51] Kattan S. W., Nafie M. S., Elmgeed G. A., Alelwani W., Badar M., Tantawy M. A., Molecular docking, anti-proliferative activity and induction of apoptosis in human liver cancer cells treated with androstane derivatives: Implication of PI3K/AKT/mTOR pathway. *The Journal of Steroid Biochemistry Molecular Biology*. **198**, 105604 (2020)..

[52] Tantawy M. A., Sroor F. M., Mohamed M. F. El-Naggar M. E., Saleh F. M., Hassaneen H. M. Abdelhamid I. A. Molecular Docking Study, Cytotoxicity, Cell Cycle Arrest and Apoptotic Induction of Novel Chalcones Incorporating Thiadiazolyl Isoquinoline in Cervical Cancer. *Anticancer Agents Medicinal Chem*istry, **20**, 70-83 (2019).

[53] Nafie M. S., Tantawy M. A., Elmgeed G. A., Screening of different drug design tools to predict the mode of action of steroidal derivatives as anti-cancer agents. *Steroids*. **152**, 108485 (2019).

[54] Vichai V., Kirtikara K., Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature Protocols*. **1** (3), 1112-1116 (2006).

[55] Li L. H., Yu F. L., Transcriptional specificities of adriamycin. *Biochemistry and Molecular Biology International*, **31** (5), 879-887 (1993).

[56] Abdel-Motaal M., Almohawes K., Tantawy M. A. Antimicrobial evaluation and docking study of some new substituted benzimidazole-2yl derivatives. *Bioorganic Chemistry*. **101**, 103972 (2020).

[57] El-Far A. H., Tantawy M. A., Al Jaouni S. K., Mousa S. A., Thymoquinone-chemotherapeutic combinations: new regimen to combat cancer and cancer stem cells. *Naunyn Schmiedebergs Archives Pharmacology*, **393** (9), 1581-1598 (2020).

[58] Tantawy M. A., El-Sherbeeny N. A., Helmi N. Alazragi R. Salem N. Elaidy S. M. Synthetic antiprotozoal thiazolide drug induced apoptosis in colorectal cancer cells: implications of IL-6/JAK2/STAT3 and p53/caspases-dependent signaling pathways based on molecular docking and in vitro study. *Molecular and Cellular Biochemistry*, **469** (1-2), 143-157 (2020).

مشتقات ميلاتونين مبتكرة: تشييد ، تقييمات مضادة للسرطان ودراسة الالتحام الجزيئي

محمود السيد محمود صلاح الدين 1 ، دينا سعيد ابواليزيد القاضى2، محمد عبد الحميد طنطاوى2، ميرفت محمود عبد الحليم حسن 2، سامية رضوان العزباوى1، أميرة السيد محمودعبد الله 1، جمال عبد المجيد عبد الغنى2

1 قسم الكيمياء ، كلية العلوم ، جامعة حلوان، عين حلوان ،القاهرة، مصر
2 قسم الهرومونات، شعبة البحوث الطبية، المركز القومى للبحوث ، الدقى ، الجيزة ، مصر

ذكرت العديد من الدراسات أن الميلاتونين يعتبر عاملًا مضادًا للسرطان. لذلك في هذه الدراسة، تم تشييد بعض من مشتقات الميلاتونين الجديدة والتي تحتوي على أنظمة حلقية غير متجانسة مثل التريازول ، الثياديازول ، التترازول ، الثيازول ، الثيوفين ، والبيرازول. تم تقييم المركبات المحضرة 5 و 6 و 8 و 11 و 15 و 16 و 19 كمضادات للسرطان باستخدام نوعين من الخلايا السرطانية البشرية هم سرطان الثدي(MCF7) وسرطان القولون(HCT-116). حيث أظهرت هذه المركبات انخفاضًا تدريجيًا في نمو هذه الخلايا. لاحظنا أيضًا أن المركب 16 كان أقل المركبات تركيزا و أعلى سمية على نوعين الخلايا السرطانية التي تم أختبارها. علاوة على ذلك ، فقد تمت دراسة الالتحام الجزيئي لتحديد مدى فاعلية المركبات المشيدة ضد البروتينات (CDK2) و(P53-MDM2) والتى تعتبر بروتينات لها أثر محتمل فى التسبب بالسرطان. وقد كشفت تحليلات الالتحام الجزيئي أن المركب 16 كان أفضل مركب ضد البروتينات المستهدفة ، حيث أظهر أقل طاقات الالتحام والأرتباط عبر الروابط الهيدروجينية ، والتفاعلات الكارهة للماء مقارنة بالمركبات الأخرى المشيدة.