WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 16, 22-43. Research Article ISSN 2277– 7105

SYNTHESIS, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF SOME NEW CHALCONES INCORPORATING INDOLE BASED-MELATONIN ANALOGUES

Nadia T. A. Dawoud*[1], S. M. Mohamed[2], Doaa M. M. Elbadawy[3], Fatma S. M. Moawed[4] and Amel F. M. Ismail[5]

[1,2,3]Department of Chemistry, Faculty of Science(Girls), AL-Azhar University, Nasr City, Cairo, Egypt.

[4]Health Radiation Research Department, National Center for Radiation Research and Technology(NCRRT), Atomic Energy Authority, Nasr City, Cairo, Egypt.

[5]Drug Radiation Research Department, National Center for Radiation Research and Technology(NCRRT), Atomic Energy Authority, Nasr City.

Article Received on
03 July 2018,

Revised on 23 July 2018,
Accepted on 12 August 2018

DOI: 10.20959/wjpr201816-13141

***Corresponding Author**
Nadia T. A. Dawoud
Department of Chemistry, Faculty of Science(Girls), AL-Azhar University, Nasr City, Cairo, Egypt.

ABSTRACT

Melatonin (MLT) has been indicated as a possible oncostatic agent in different types of cancer, its anti-proliferative role being demonstrated in several in-vitro and in vivoexperimental models of tumors. Research on MLT related compounds has been required to optimize the maximum pharmaceutical activity with the lowest side effects. In this study a series of some new 3-(substitutedaryl)-N-(5-methoxy-1H-indol-3-yl)ethyl) acryl amide (**2a-e**) has been synthesized and characterized on the basis of elemental analysis, spectral data (IR,^{1}H-, ^{13}C-NMR and Mass spectra). In-vitro antioxidant activity of the newly synthesized compounds was investigated by evaluating their radical scavenging activity via the DPPH assay. In addition, the cytotoxic activity of the newly synthesized compounds was evaluated against human hepatocellular carcinoma HepG2 cells. Compounds (**2c, 2d,2e**) exhibited a potent in-vitro cytotoxic activity than the reference drug doxorubicin, so significant implications on future clinical exploitation of these molecules in hepatocellular cancer therapeutics may be occurred.

KEYWORDS: Melatonin, Chalcones, Antioxidant activity, Cytotoxicity, HepG2.

1. INTRODUCTION

N-acetyl-5-methoxytryptamine, or melatonin(MLT) is an indolic hormone which plays pleiotropic roles and is widely distributed in most living organisms, where it is involved in various physiological functions.[1,3] MLT is a hormone primarily secreted by the pineal gland along with many other parts of the human body.[4,7] The relevance of melatonin has been demonstrated in human physiology and pathology, due to its anti-inflammatory properties, antioxidant action, and its role in immunomodulation, energy metabolism, and hematopoiesis. Furthermore, a number of epidemiological studies support a protective role of melatonin in both hormone-dependent and hormone-independent cancers, suggesting an inverse correlation between melatonin level and cancer incidence.[8,9]

In recent years, the many physiological properties of MLT have stimulated much interest in the development of synthetic compounds possessing the indole ring. The diverse biological activities of melatonin may be attributed to the presence of C—NH—C fragment, characteristics of the indole ring. Recent research has showed that compounds with an indole ring have significant antioxidant effects.[10]

Chalcones are naturally occurring α,β -unsaturated ketones with two aromatic rings (A and B) belonging to the flavonoid family which constitutes one of the major classes of naturally occurring oxygen heterocyclic compounds.[11] In nature, (*E*)-chalcones have shown to display an important role in pigmentation of flowers and can act as protecting agents against micro-organisms, insects and ultraviolet radiation. They can also present other biological, pharmacological and biocidal properties, such as antibacterial[12], anti-inflammatory[13,15], antifungal[12], antimalarial[16,18], antitumor[19], antimicrobial[20,21], antiviral[22], antitubercular[23], antioxidant[24], antimitotic[25], antileishmanial[26], antiplatelet[27], anticancer[28] activities, among others.[29,30]

Herein, we report the synthesis of some novel chalcones incorporating indole-based melatonin analogue for evaluation to antioxidant and cytotoxic activities against human hepatocellular carcinoma HepG2cell line.

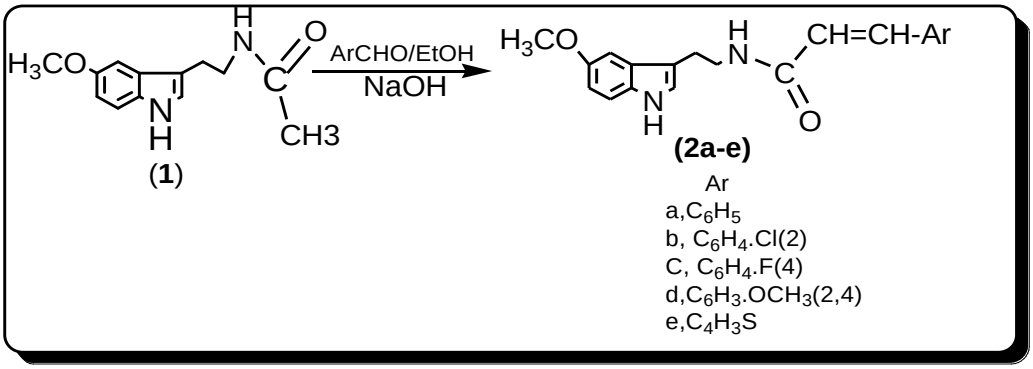
2. RESULTS AND DISCUSSION

2.1. Chemistry

Aconsiderable variety of methods are available in literature for the synthesis of chalcones. The most convenient method is the one, that involves the Claisen-Schimidt condensation of

equimolar quantities of an aryl methyl ketones with arylaldehyde in presence of alcoholic alkali.[31]

Thus, treatment of ethanolic solution of [Melatonin] (2-(5-methoxy-1H-indol-3-yl)ethyl) acetamide(**1**) with aromatic aldehydes namely, benzaldehyde, 2-chlorobenzaldehyde, 4-flourobenzaldehyde, 2,4-dimethoxy benzaldehyde and/or thiphenaldehyde in the presence of sodium hydroxide as a catalyst(Clasien-Sehmidt reaction conditions[32,34] affected condensation with the formation of the corresponding N-(2-(5-methoxy-1H-indol-3-yl) ethyl) Cinnamamide(**2a**) and/or 3-(substituted aryl)-N-(5-methoxy-1H-indol-3-yl)ethyl) acrylamide(chalcones) (**2b-d**)(Scheme 1)**,** respectively.



Scheme 1

The structures of compounds (**2a-e**) were supported by elemental analysis and spectral data. The IR spectra of (**2a-e**) showed strong absorption bands at 3349-3255 and 1675-1651cm^{-1} attributed to NH and CO of α-β unsaturated carbonyl group whereas, the ^{1}H-NMR spectra revealed singlet broad signals at δ 10.65-10.63, 8.40-7.95ppm integrating for two NH groups of melatonin moiety which is D_2O-exchangeable, pair doublet of two protons at δ 6.70, 7.85ppm integrating for α and β–unsaturated carbonyl group. Also, a multiplet signal at 7.45-6.69 corresponding to the aryl protons and indole moiety. In addition, one singlet at δ 3.74,3.76ppm of three protons integrating for methoxyl group and disappearance of signal of CH_3 groupand this explain the conversion of $COCH_3$ of MLT into α-β unsaturated carbonyl group. All other signals are in agreement with the proposed structures.

2.2. Antioxidant activity

In the present study, *in-vitro* Anti-oxidant activity of melatonin and novel N-(2-(5-methoxy-1H-indol-3-yl) ethyl) Cinnamamide(**2a**) and/or 3-(substituted aryl)-N-(5-methoxy-1H-indol-3-yl)ethyl)acrylamide (chalcones) (**2b-d**)(chalcone derivatives) was performed by using

DPPH method.[35] It is an ideal and convenient method to assess the antioxidant activity of novel compounds. DPPH is an unstable free radical that gets maximum absorbance at 517 nm due to the presence of an odd electron, which disappears when paired with an antioxidant or reducing agent. An antioxidant or reducing agent may supply electron or hydrogen to the DPPH free radical and bleach the color of the DPPH solution. Generally, 2,2 diphenyl-1-picrylhydrazyl has produced a black purple color in methanol, it becomes colorless or yellow in color when paired with an electron and turns to 2,2 diphenyl-1-picrylhydrazyl.

Inhibitions of all the tested synthesized compounds are listed in Table 1. The antioxidant activity is expressed in terms of % inhibition and IC_{50} (effective concentration for scavenging 50% of the initial DPPH) value (µM).

Table 1: Antioxidant activity of the newly synthesized compounds (%inhibition).

Comp. Code	Concentration, µg/mL								IC_{50} µg/mL
	5	**10**	**20**	**40**	**80**	**160**	**320**	**460**	
11	1.68	4.08	7.96	14.55	24.50	33.72	45.24	55.21	464.1
2a	3.87	6.18	11.94	25.97	34.97	46.70	56.23	80.42	215.4
2b	1.99	3.98	6.70	16.65	23.77	33.61	45.76	58.01	430.8
2c	2.30	4.08	9.32	16.02	25.34	34.45	46.49	57.80	419.3
2d	3.35	4.92	7.33	21.68	29.63	40.52	53.61	78.85	275.9
2e	1.68	2.72	6.07	9.84	18.85	27.85	40.31	50.68	619
Standard	Concentration, µg/mL								IC_{50} µg/mL
	5	10	15	20	25	30	35	80.	
Ascorbic acid	12.98	16.38	62.98	76.81	78.72	78.94	80.21		11.2

Of all the compounds tested synthesized show that compounds **2a-e** showed scavenging activity towards DPPH. Compounds **2a** and **2d** showed strong inhibition than the parent material(Melatonin) but lower than the specific inhibitor AA.

2.3 In-vitro cytotoxicity against human hepatocellular carcinoma HepG2 cells

Cancer is the leading cause of worldwide morbidity and mortality, especially in developing countries.[36,38] Hepatocellular carcinoma is one of the common global malignancies and is the fifth most common type of cancer and the third leading cause of cancer-associated mortality worldwide, and the incidence continues to increase in numerous countries.[39,42] The anthracycline doxorubicin is a chemotherapeutic drug to treat different diversities of cancers.[43,44]

Most of the clinically used antitumor agents possess significant cytotoxic activity in cell culture systems as cytotoxicity is one of chemotherapeutic targets of antitumor activity.[45]

The cytotoxic effect of the newly synthesized compounds was evaluated against HepG2 cells, using MTT assay, in comparison with doxorubicin as a reference drug. The cytotoxic activities of the newly compounds are expressed by median growth inhibitory concentration (IC_{50}), whereas, cell viability percent and Toxicity percent of HepG2 cells were evaluated. The tumor cell line showed normal growth in the culture system and DMSO did not seem to have any noticeable effect on cellular growth. A gradual decrease in the viability of cancer cells was observed with increasing concentration of the tested compounds, in a dose-dependent inhibitory effect.

In-vitro anticancer activity of melatonin and doxorubicin against HepG2 cells is demonstrated in Table 2. Fig. 1 & 2 demonstrated the Cell Viability% and Toxicity% of Melatonin (1) and Doxorubicin at differentconcentrations (µM/ml) on HepG2 cells. The results showed that MLT exhibited cytotoxic activity against HepG2 cells in a concentration dose dependent manner, with IC_{50} 4.8 $\pm$ 0.20 µM/ml after 24 h of incubation, as compared to the reference drug; doxorubicin (IC_{50} 21.62 $\pm$ 0.65 µM/ml). It is obvious that MLT possess a potent in-vitro cytotoxic activity than the reference drug, doxorubicin. Fig.3,4 and 5 represented morphological changes in untreated HepG2 cells, HepG2 cells treated with different concentrations of MLT and HepG2 cells treated with different concentrations of doxorubicin, respectively. Treated HepG2 cells with MLT or doxorubicin showed partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation.

Several clinical trials have examined the therapeutic usefulness of melatonin in different types of cancer. Over the last decades, significant research efforts have been devoted to defining the role of melatonin in the pathogenesis of cancer and its possible therapeutic application. It has been shown that melatonin, under bothin-vitro and in vivo conditions, inhibits the growth of some cancer cells.[46] Melatonin induced antineoplastic activity in both in-vivo and in-vitro models of carcinogenesis.[47] In this in-vitro study, melatonin performs as a cytotoxic agent against human liver adenocarcinoma. These data are in bargain with previous states on a neoplasms ranges, including, breast cancer[48,54], prostate cancer cells[55,58], melanoma[59], leukemia[60], colon cancer[61,63], ovarian carcinoma cell line[64], neuroblastoma[65], hepatocarcinoma cell lines.[66,67]

Melatonin exerts growth inhibitory effects on different cancer cell lines. Melatonin may be exerts growth inhibitory effects through its activation of MT1 and MT2 receptors, and inhibition of linoleic acid uptake, thereby preventing the formation of the mitogenic

metabolite 1,3-hydroxyoctadecadienoic acid. Further, the anti-carcinogenic effect of melatonin on neoplastic cells relies on its antioxidant, immune-stimulating, and apoptotic properties.[68,70] MT2 expression is absent in HepG2 cells, so the cytotoxic effect of melatonin is driven through MT1 in HepG2 cells, which is correlated to cAMP modulation and ERK activation by melatonin.[71] Also, melatonin induced cytotoxic effect on HepG2 mediated by induction of apoptosis, cell death and cell cycle arrest,[66,67,71]

On the other hand, anticancer activities of the newly synthesized compounds(**2a-e**) against liver adenocarcinoma HepG2 cellsare listed in Table 3. Fig. 6 & 7 displayed the Cell Viability% and Toxicity% of different concentrations (µM/ml) of the newly synthesized compounds on HepG2 cells. Fig 8, 9, 10,11 and 12 represented the morphological changes in HepG2 cells treated with different concentrations of the newly synthesized compounds. HepG2 cells treated with the newly synthesized compounds also showed partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation.

The obtained data in Table 3 revealed the in-vitro anti-cancer activity direction of the newly synthesized compounds against HepG2 is **2d**>**2c**>**2e**>**2b**>**2a** with IC_{50}: 9.43 $\pm$ 0.45 > 17.81 $\pm$ 0.80 > 18.18 $\pm$ 0.60 > 27.34 $\pm$ 0.75 > 54.23 $\pm$ 3.95µM/ml, respectively as compared to the reference drug; doxorubicin (IC_{50}: 21.62 $\pm$0.65µM/ml).

A closure look into the structure activity relationship indicates that the melatonin derivatives **2c**, **2d** and **2e** which bearing 3-(4-fluoro-phenyl), 3-(2,4-dimethoxyphenyl) and 3-thiophen-3-yl moieties, respectively with an IC_{50} value of : 9.43 $\pm$ 0.45 > 17.81 $\pm$ 0.80 > 18.18 $\pm$ 0.60 > 27.34 $\pm$ 0.75 > 54.23 $\pm$ 3.95µM/ml exhibited more potent in-vitro cytotoxic activitiesthanthe reference drug; doxorubicin (IC_{50}: 21.62 $\pm$0.65µM/ml) but showed less in-vitro cytotoxic activity than their parent MLT (IC_{50}: 4.8 $\pm$ 0.20 µM/ml). The cytotoxic mechanism of these newly synthesized compounds will beinvestigated in a further study.

Table 2: In-vitro anticancer activity of various concentrations of melatonin and doxorubicin against human liver adenocarcinoma HepG2 cells as assessed with MTT assay method.

Compound	Concentration (µM/ml)	Viability % (± St. D.)	Toxicity % (± St. D.)	IC_{50}% (± St. D.) (µM/ml)
HepG2 Cells		100.00 ± 0.20	0.00 ± 0.00	
Doxorubicin (Dox)	100	4.44 ± 0.81	95.55 ± 0.81	**21.62 ± 0.65**
	50	7.36 ± 1.52	92.64 ± 1.52	
	25	13.34 ± 1.53	86.66 ± 1.53	
	12.5	43.52 ± 0.50	56.48 ± 0.50	
	6.25	66.19 ± 0.80	33.81 ± 0.80	
	3.125	96.39 ± 0.65	3.61 ± 0.65	
	1.562	98.97 ± 1.04	1.03 ± 1.04	
	0.781	100.76 ± 0.67	00 ± 00	
	0.390	101.20 ± 1.06	00 ± 00	
	0.195	103.11 ± 1.17	00 ± 00	
1 (Melatonin)	100	3.67 ± 0.80	96.33 ± 0.80	**4.8 ± 0.20** [a]
	50	10.76 ± 0.86	89.24 ± 0.86	
	25	13.95 ± 0.56	86.04 ± 0.56	
	12.5	15.12 ± 0.85	84.88 ± 0.85	
	6.25	27.16 ± 0.80	72.84 ± 0.80	
	3.125	66.19 ± 0.90	33.81 ± 0.90	
	1.562	97.29 ± 0.70	2.71 ± 0.70	
	0.781	99.72 ± 0.63	0.33 ± 0.58	
	0.390	102.06 ± 2.08	0.00 ± 0.00	
	0.195	103.20 ± 1.70	0.00 ± 0.00	

* Data are expressed as means ± S.D. of three separate experiments.

**Median growth inhibitory concentrations (IC_{50}).

***[a]Indicates a significant difference from doxorubicin at $p < 0.05$

Table 3: In- vitro anticancer activity of the newly synthesized compounds against human liver adenocarcinoma HepG2 cells.

Compound	Concentration (µM/ml)	Viability % (± St. D.)	Toxicity % (± St. D.)	IC_{50}% (± St. D.) (µM/ml)
2a	100	5.04 ±0.26	94.95 ± 0.27	**54.23 ± 3.95**
	50	38.14 ± 0.75	61.85 ± 0.75	
	25	87.79 ± 0.75	12.21 ± 0.75	
	12.5	100.40 ± 0.69	0.00 ± 0.00	
	6.25	100.22 ± 0.38	0.00 ± 0.00	
	3.125	102.31 ± 2.07	0.00 ± 0.00	
2b	100	6.16 ± 0.60	93.84 ± 0.6	**27.34 ± 0.75**
	50	19.24 ± 0.65	80.75 ± 0.65	
	25	45.74 ± 0.60	54.26 ± 0.60	
	12.5	51.37 ± 0.64	48.63 ± 0.64	

	6.25	99.43 ± 1.21	0.83 ± 0.76	
	3.125	99.62 ± 0.41	0.37 ± 0.40	
	1.562	99.02 ± 0.69	0.97 ± 0.96	
	0.781	101.13 ± 1.91	0.00 ± 0.00	
2c	100	5.59 ± 0.52	94.41 ± 0.52	**17.81 ± 0.80[a]**
	50	9.02 ± 0.45	90.97 ± 0.45	
	25	27.44 ± 0.45	72.56 ± 0.45	
	12.5	60.60 ± 1.08	39.40 ± 1.08	
	6.25	96.45 ± 0.68	3.55 ± 0.68	
	3.125	99.42 ± 1.38	0.90 ± 0.85	
	1.562	100.35 ± 1.51	0.32 ± 0.55	
2d	100	5.36 ± 0.41	94.64 ± 0.40	**9.43 ± 0.45[a]**
	50	6.16 ± 0.50	93.84 ± 0.50	
	25	9.33 ± 0.35	90.67 ± 0.35	
	12.5	20.16 ± 0.65	79.83 ± 0.65	
	6.25	82.68 ± 0.62	17.31 ± 0.61	
	3.125	101.09 ± 3.76	0.67 ± 1.15	
	1.562	99.60 ± 1.44	0.67 ± 1.15	
	0.781	100.10 ± 0.66	0.17 ± 0.28	
2e	100	4.32 ± 0.62	95.68 ± 0.62	**18.18 ± 0.60[a]**
	50	13.15 ± 1.04	86.85 ± 1.04	
	25	21.71 ± 1.75	78.29 ± 1.75	
	12.5	74.38 ± 1.09	25.62 ± 1.09	
	6.25	97.30 ± 1.13	2.69 ± 1.13	
	3.125	100.09 ± 1.14	0.34 ± 0.58	
	1.562	98.57 ± 0.52	1.43 ± 0.52	
	0.781	100 ± 1.0	00 ± 00	

* Data are expressed as means ± S.D. of three separate experiments.

Median growth inhibitory concentrations (IC_{50}) *[a]indicates a significant difference from doxorubicin at $p < 0.05$.

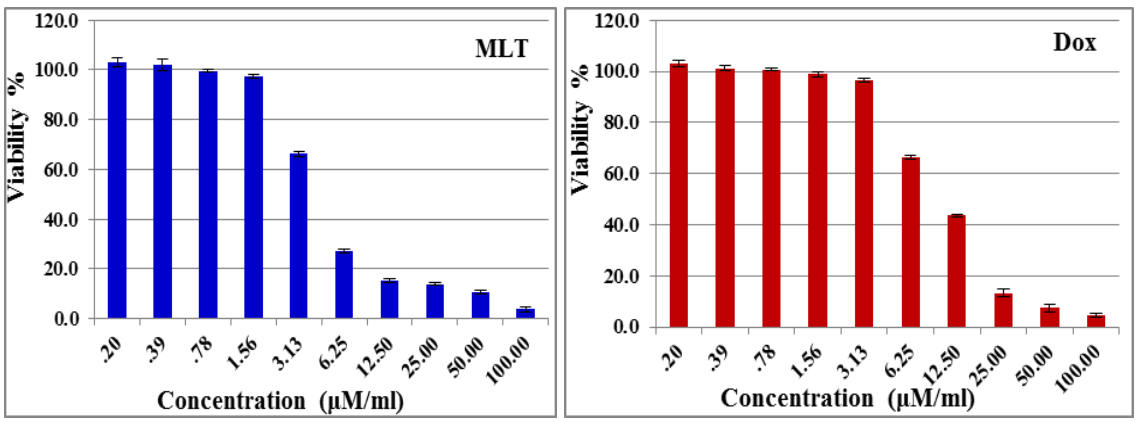


Fig. 1: Cell Viability% of different Melatonin (1) and Doxorubicin Concentrations (µM/ml) on liver adenocarcinoma HepG2 cells.

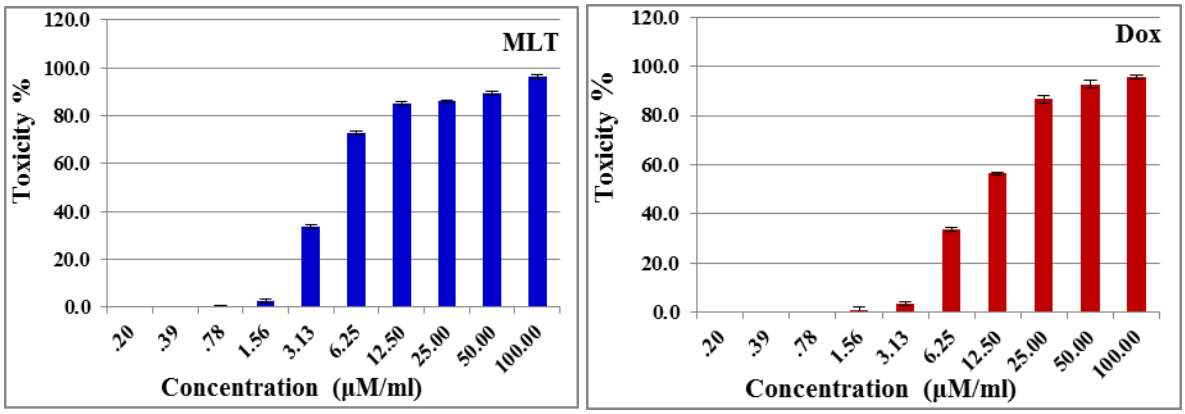


Fig. 2: Toxicity% of different Melatonin (1) and Doxorubicin Concentrations (µM/ml) on liver adenocarcinoma HepG2 cells.

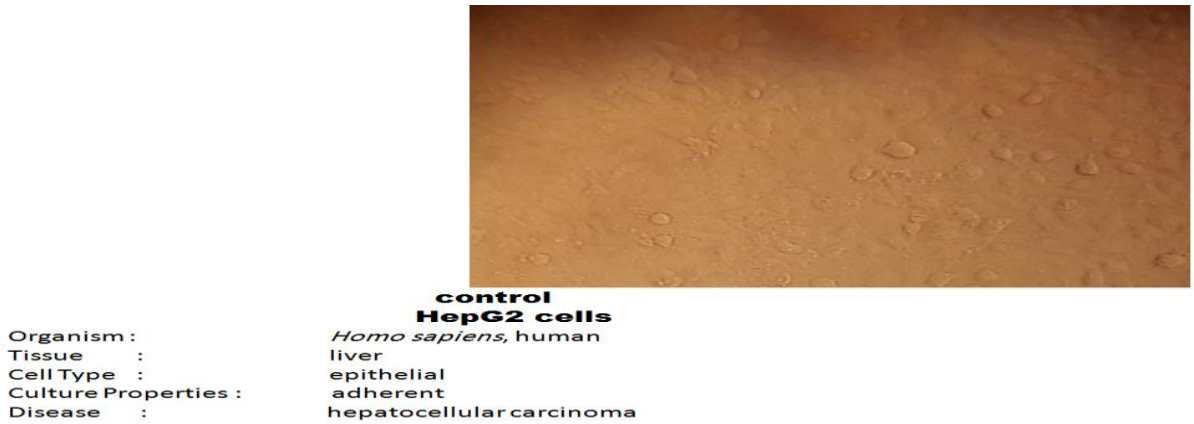


Fig. 3: Human liver adenocarcinoma HepG2 cells.

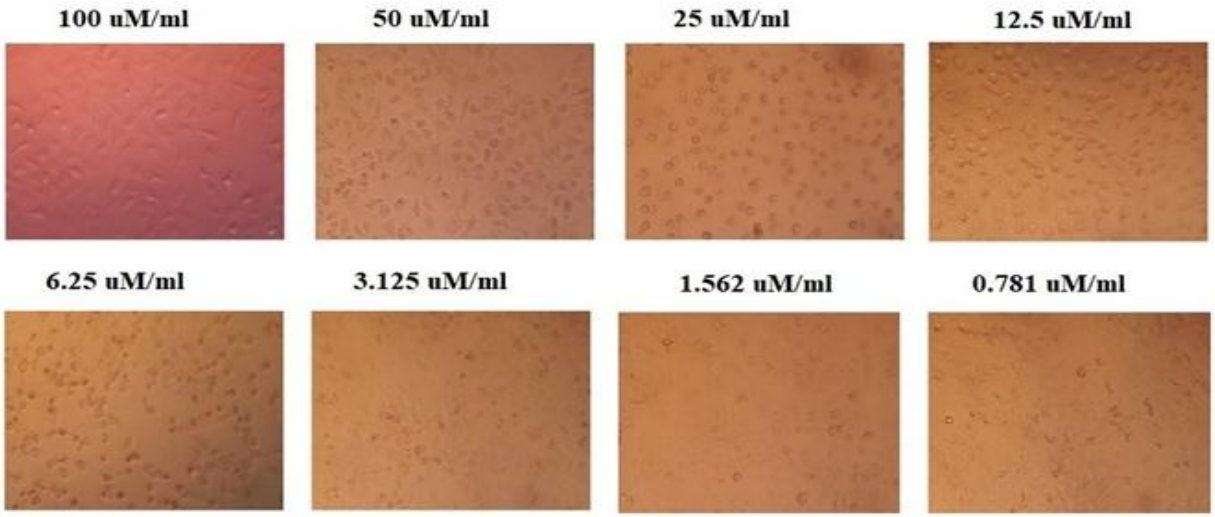


Fig. 4: Effect of different concentrations of compound Melatonin (N-(2-(5-methoxy-1H-indol-3-yl)ethyl)acetamide) (1) on human Liver adenocarcinoma HepG2 cells.

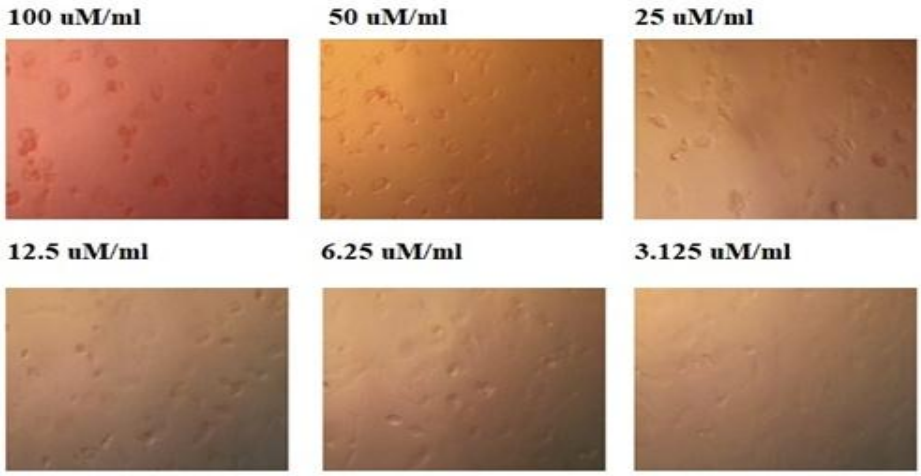


Fig. 5: Effect of different concentrations of Doxorubicin (reference drug) on human liver adenocarcinoma HepG2 cells.

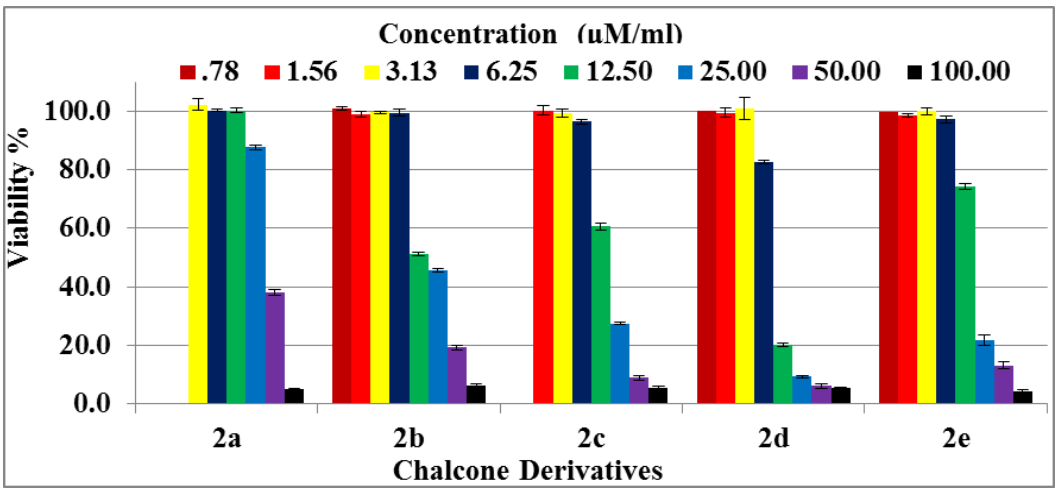


Fig. 6: Cell Viability% of the newly synthesizedchalcone derivatives of Melatonin on human liver adenocarcinoma HepG2 cells.

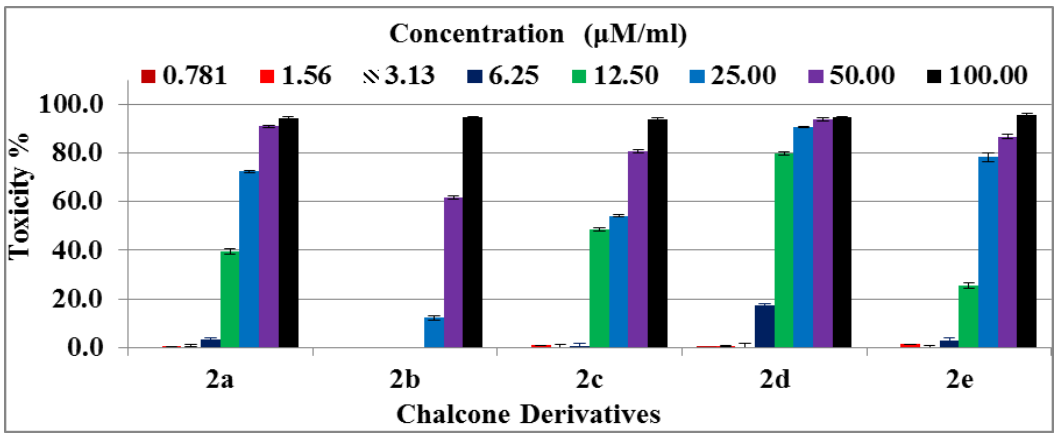


Fig. 7: Toxicity% of the newly synthesizedchalcones of Melatonin (2a-e) on human liver adenocarcinoma HepG2 cells.

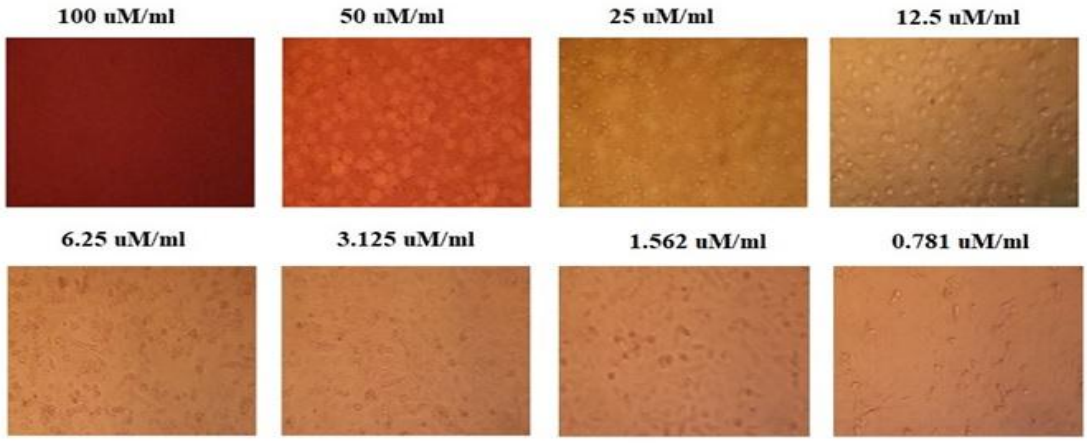


Fig. 8: Effect of different concentrations of compound N-(2-(5-methoxy-1H-indol-3-yl) ethyl) cinnamide(2a) on human liver adenocarcinoma HepG2 cells.

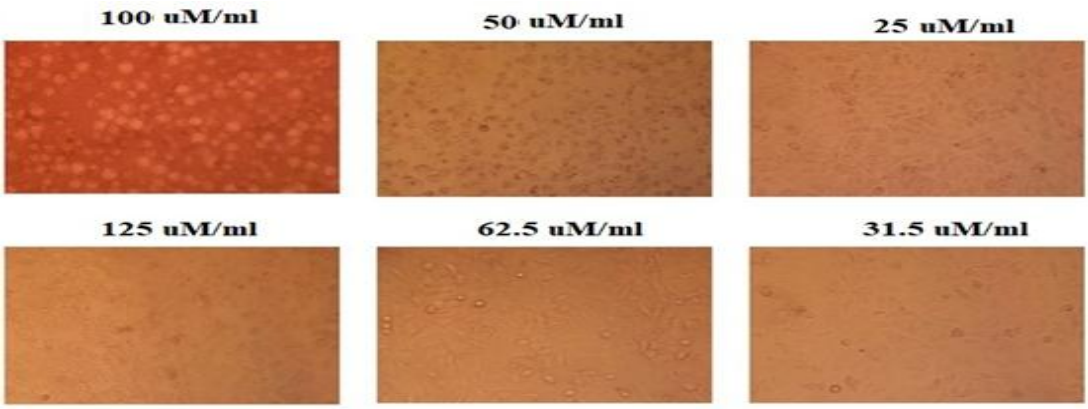


Fig. 9: Effect of different concentrations of compound 3-(4-chlorophenyl) N-(2-(5-methoxy1H-indol-3-yl)ethyl) acrylamide(2b) on human liver adenocarcinoma HepG2 cells.

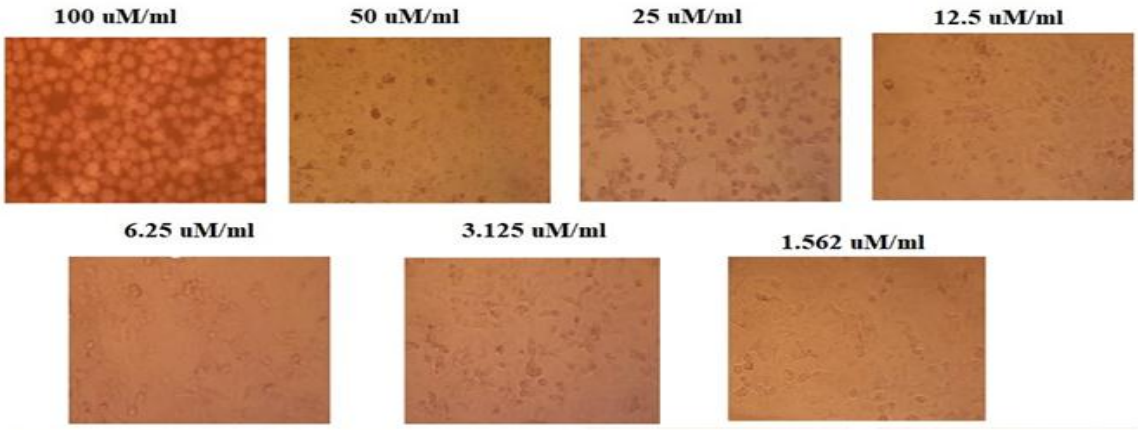


Fig. 10: Effect of different concentrations of compound (E)- 3-(4- fluorophenyl) -N-(5-methoxy-1H-indol-3-yl)ethyl)acrylamide(2c) on human liver adenocarcinoma HepG2 cells.

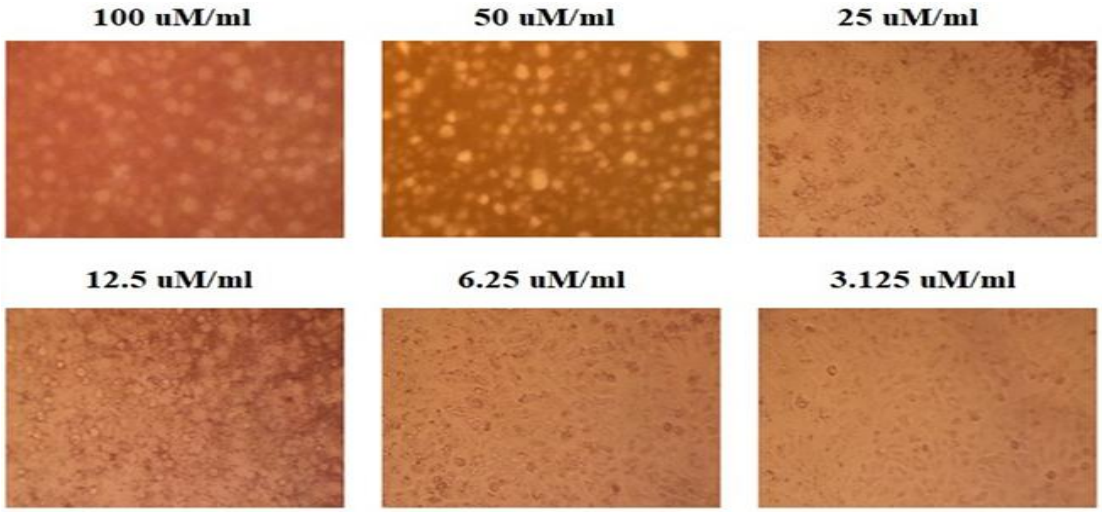


Fig. 11: Effect of different concentrations of N-(2-(5-methoxy-1H-indol-3-yl)ethyl)-3-(2,4-dimethoxyphenyl)acrylamide (2d) on human liver adenocarcinoma HepG2.

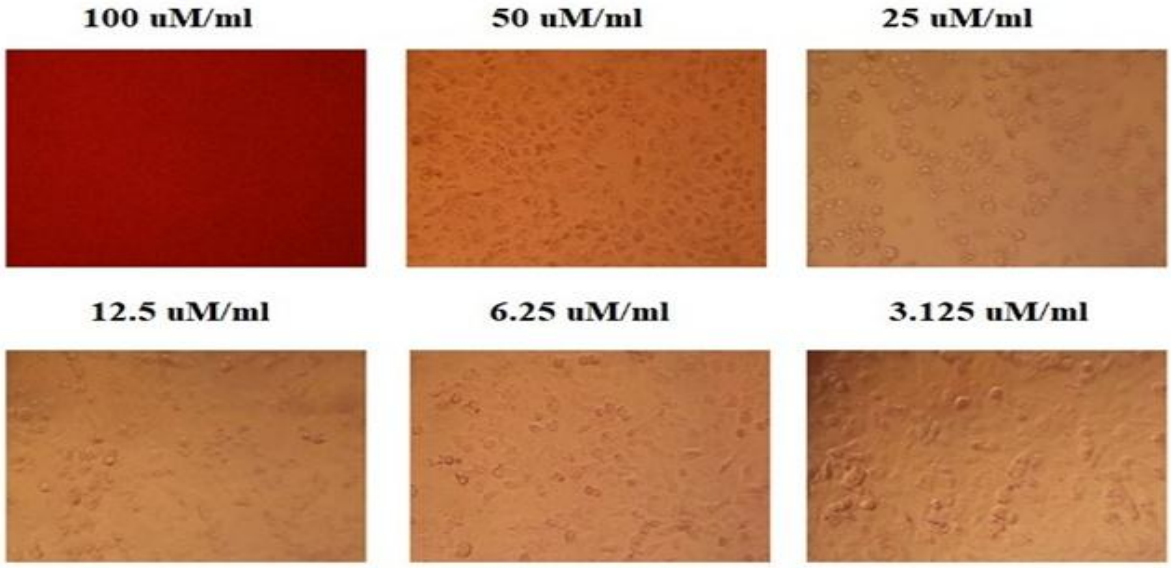


Fig. 12: Effect of different concentrations of compound (E) -N-(2-(5-methoxy-1H-indol-3-yl)ethyl)-3-(thiophen-3-yl) acrylamide(2e) on human liver adenocarcinoma HepG2 cells.

3. CONCLUSION

In summary, we had synthesized a novel series of chalcones of indole-based on melatonin analogues. Most of the synthesized showed scavenging activity towards DPPH. Compounds **2a** and **2d** showed strong inhibition than the parent material (MLT) but lower than the specific inhibitor AA. Also, the newly synthesized compounds were evaluated against human liver adenocarcinoma HepG2 cells. Most of the prepared compounds revealed potential cytotoxic anticancer activity. Compounds **2c**, **2d** and **2e,** which bearing 3-(4-Fluoro-phenyl),

3-(2,4-dimethoxyphenyl) and 3-thiophen-3-yl moieties with an IC_{50} value of : 9.43 ± 0.45 > 17.81 ± 0.80 > 18.18 ± 0.60 > 27.34 ± 0.75 > 54.23 ± 3.95µM/ml were exhibited more potent in-vitro cytotoxic activitiesthan the reference drugdoxorubicin (IC_{50}: 21.62 ±0.65µM/ml), but showed less active in-vitro cytotoxic activities than their parent material (melatonin) (IC_{50}: 4.8 ± 0.20 µM/ml). The cytotoxic mechanism of these newly synthesized compounds will be investigated in a further study.

4. Experimental

All melting points for the prepared derivatives were measured in capillary tubes using a Gallen-Kamp apparatus and were uncorrected. The FT-IR spectra were recorded on a Perkin-Elmer 1650 spectrophotometer (KBr pellets). The 1H,13C NMR spectra were measured in dimethylsulphoxide-*d*6 as a solvent using a Varian Gemini 180 spectrometer operating at 400 MHz for 1H and 75 MHz for 13C NMR. TMS was used as an internal standard and the chemical shifts were reported as δ ppm. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer.

4.1 General procedure

Synthesis of N-(2-(5-methoxy-1H-indol-3-yl) ethyl)cinnamamide (2a) and/or 3-(substituted aryl)-N-(5-methoxy-1H-indol-3-yl)ethyl)acrylamide(2b-e)

To ethanolic solution of melatonin **1** (0.01 mol) containing (5 mL) of 10% aqueous NaOH, aromatic and hetero aldehydes namely, (benzaldehyde, 2-chlorobenzaldehyde, 4-flouro benzaldehyde, 2,4-dimethoxybenzaldehyde and /or thiophenaldehyde (0.01 mol) were added. The reaction mixture was heated under reflux for 6-8 hrs. the solvent was concentrated, left to cool. Acidified with acetic acid to give (**2a-e**), respectively.

N-(2-(5-methoxy-1H-indol-3-yl) ethyl) cinnamamide(2a)

$C_{20}H_{20}N_2O_2$ (MW320): orange crystals; 76% yield; m. p 80 -82 ^oC. Anal Calc: C,75.01; H, 6.31; N, 8.77; Found: C, 74.98; H, 6.29; N,8 .77. FT-IR (KBr cm-1)υ max: 3285 (NH), 3070(CH-aromatic), 2911.2863 (CH-aliphatic),1665(CO), 1596(C=C). 1HNMR (δ ppm) (DMSO-*d*6): δ=10.64(1H,s,br,NHindole),8.29(1H,s,br,NHCO), 7.73(1H,d,CH= CHCO), 6.71 (1H,d, CH=CHCO), 6.69-7.45(9H,m,H-Ar),3.86(2H,t,CH_2CH_2NH). 3.75(3H,s,OCH_3), 2.79(2H,t,CH_2 CH_2NH).

3-(Chloro phenyl)-N-(5-methoxy-1H-indol-3-yl)ethyl) acrylamide(2b)

$C_{20}H_{19}ClN_2O_2$(MW354): brownish crystals; 65% yield; m. p 72 -74 ^oC. Anal Calc: C,67.72; H, 5.45; N, 8.02;Cl,10.01; Found: C, 67.70; H, 5.40; N,7.89;9.99. FT-IR (KBr cm-1)υ max:3349 (NH),3062(CH-aromatic), 2906-2898 (CH-aliphatic), 1651(CO), 1576(C=C), 610(C-Cl). 1HNMR (δ ppm) (DMSO-*d6*): δ =10.65(1H,s,brNH indole), 7.95(1H,br,NHCO), 7.55(1H,d,CH=CHCO), 7.02-7.41(8H,m,H-Ar), 6.70(1H,d,CH =CHCO), 3.76(3H,s,OCH_3), 3.30 (2H,q,(CH_2CH_2NH), 2.76(2H,t,CH_2CH_2NH).

3-(Flouro phenyl)-N-(5-methoxy-1H-indol-3-yl)ethyl) acrylamide(2c)

$C_{20}H_{19}FN_2O_2$ (MW388):dark brown crystals; 72% yield; m. p 93 -95^oC. Anal Calc: C, 80.01; H, 5.68; N, 8.31; F,5.63; Found: C, 77.99; H, 5.66; N,8.28,F;5.61. FT-IR (KBr cm-1)υ max:3255 (NH),3035(CH-aromatic), 2961-2916 (CH-aliphatic), 1647(CO), 1604 (C=C),799(C-F). 1HNMR (δ ppm) (DMSO-*d6*): δ =10.63(1H,d,NH-indole), 8.20 (1H,s,br,NHCO),7.85(1H,d,CH=CHCO),7.02-7.24(8H,m,H-Ar), 6.95(1H,d,CH= CHCO), 3.74(3H,s,OCH_3), 3.29 (2H,q,(CH_2CH_2NH), 2.50(2H,t,CH_2CH_2NH). MS: *m/z* (%), 338(M^+ 1%); 160 (100%).

3-(2,4-Dimethoxy phenyl)-N-(5-methoxy-1H-indol-3-yl)ethyl) acrylamide(2d)

$C_{22}H_{24}N_2O_4$ (MW380): brownish red crystals; 65% yield; m. p 70 -72^oC. Anal Calc: C, 69.48; H,6.39; N,7.38; Found: C, 69.46; H, 6.36; N,7.36. FT-IR (KBr cm^{-1})υ max: 3271 (NH),3070(CH-aromatic), 2935-2831 (CH-aliphatic), 1659(CO), 1612 (C=C). MS: *m/z* (%), 382 (M^{+2} 24%); 310(100).

N-(2-(5-methoxy-1H-indol-3-yl)ethyl)-3-(thiophen-2-yl)acrylamide(2e)

$C_{18}H_{18}N_2O_2S$ (MW326): brownish crystals; 82% yield; m. p 104-106^oC. Anal Calc: C, 66.30; H,5.58; N,8.61;S,9.87; Found: C, 66.23; H,5.56; N,8.58;S,9.82. FT-IR (KBr cm-1)υ max: 3302 (NH), 3082(CH-aromatic), 2989-2989 (CH-aliphatic), 1675(CO),1585 (C=C). 1HNMR (δ ppm) (DMSO-*d6*): δ = 10.65(1H,s,brNH-indole),8.40(1H,s,br, NHCO),7.63 (1H,d,CH=CHCO), -7.40(1H,d,CH=CHCO), 6.69-7.24(7H,m,H-Ar) ,3.76(3H,s,OCH_3), 3.29 (2H,q,(CH_2CH_2NH), 2.76(2H,t,CH_2CH_2NH).MS: *m/z* (%), 295(0.70%) with elimination of OCH_3; 160 (100).

4.2 Antioxidant assay

DPPH free radical scavenging activity

Freshly prepared (0.004% w/v) methanol solution of 2,2-diphenyl-1-picrylhydrzyl(DPPH) radical was prepared and stored at 10oC in the dark. A methanol solution of the test compound was prepared. A 40 µL aliquot of the methanol solution was added to 3 mL of DPPH solution. Absorbance measurements were recorded immediately with a UV-Visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

PI= [[(AC-AT)/AC] x100]]

Where AC=Absorbance of the control at t=0 min and AT=Absorbance of the sample + DPPH at t= 16 min.

4.3 Cytotoxic assay

The HepG2 human liver cell line was obtained from the American Type Culture Collection (Rockville, MD, USA). The tumor cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at the 37°C in a humidified atmosphere containing 5% CO_2. HepG2 cells at a concentration of 0.50 x 10^6 were grown in a 25 cm^2 flask in 5 ml of complete culture medium.

4.3.1 In-vitro cytotoxic assay on HepG2 cells (MTT assay)

In-vitro cytotoxic screening was evaluated at Science way for Research laboratory, Cairo, Egypt according to Mosmann (1983).[72] To determine cell viability the colorimetric MTT metabolic activity assay was used. HepG2 cells (1 × 10^5 cells/well; 100 µl) were cultured in a 96-well plate at 37°C, and exposed to varying concentrations of melatonin or each of the new prepared compounds for 24 h. HepG2 Cells treated with medium only served as a negative control group. After removing the supernatant of each well and washing twice by PBS, 20 µl of MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) solution (5mg ml^{-1} in PBS) and 100 µl of medium were then introduced. After incubation for another 4 h, the resultant formazan crystals were dissolved in dimethylsulfoxide (DMSO, 100 µl) and the

absorbance intensity measured by a microplate reader at 560 nm with a reference wavelength of 620 nm. All experiments were performed in triplicate, and the relative cell viability (%) was expressed as a percentage relative to the untreated control cells.

4.4. Statistical analysis

All statistical analyses were conducted by using the statistical package for Windows Version 21 (SPSS Software, Chicago, IL). The results for continuous variables were expressed as Means ± Standard deviation (S.D.) of three identical experiments. Values were compared by one-way analysis of variance (ANOVA). Post-hoc testing was performed for inter-group comparisons using the least significant difference (LSD) test, and significance of p values < 0.05 was considered statistically significant.

REFERENCES

1. Lerner AB, Case JD, Takahashi Y. Isolation of melatonin and 5-methoxyindole-3-acetic acid from bovine pineal glands. J Biol Chem., 1960; 235: 1992–1997.
2. Pandi-Perumal, SR, Srinivasan V, Maestroni G.J, Cardinali DP. Poeggeler, B.; Hardeland, R. Melatonin: Nature's most versatile biological signal? FEBS J., 2006; 273: 2813–2838.
3. Hardeland R, Cardinali DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR. Melatonin—A pleiotropic, orchestrating regulator molecule. Prog. Neurobiol, 2011; 93: 350–384.
4. Militante J and Lombardini JB. Age-related retinal degeneration in animal models of aging: possible involvement of taurine deficiency and oxidative stress. *Neurochem Res.*, 2004; *29*(1): 151-160.
5. Pieri C, Moroni F, Marra M, et al. Melatonin is an efficient antioxidant. *Arch Gerontol Geriatr*, 1995; *20*(2): 159-165.
6. Ressmeyer, AR, Mayo JC, Zelosko V, et al. Antioxidant properties of the melatoninmetaboliteN1-acetyl-5-methoxykynuramine (AMK): scavenging of free radicals andprevention of protein destruction. Redox Report, 2003; 8(5): 205-213.
7. Süzen S. Antioxidant activities of synthetic indole derivatives and possible activitymechanisms. *Top Heterocycl Chem © Springer-Verlag Berlin Heidelberg Published online, 5 July 2007; 11*: 145–178.
8. Calastretti G, Gatti V, Lucini S, Dugnani G, Canti F. Scaglione and AnnamariaB. Melatonin Analogue Antiproliferative and Cytotoxic Effects on Human Prostate Cancer

Cells. Int. J. Mol. Sci., 2018; 19: 1505; doi:10.3390/ijms19051505.

9. LiY, Li S, Zhou Y, Meng X, Zhang JJ, Xu DP, Li HB. Melatonin for the prevention and treatment of cancer. Oncotarget., 2017; 8: 39896–39921.
10. Sibel S, Betul Tekiner-G, Hanif Sh, Duysal U, Hande Gurer-O, Mehmet G, and Sibel Aysil On. Antioxidant activity of indole-based melatonin analogues in erythrocytes and their voltammetric characterization. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2012; Early Online: 1–13.
11. Di Carlo G, Mascolo N, Izzo AA and Capasso F. Flavonoids: Old and newaspects of a class of natural therapeutic drugs. *Life Sci.*, 1999; *65:* 337-353.
12. Siddiqui ZN, Praveen S, Musthafa TNM, Ahmad A and Khan AU. Thermal solvent-free synthesis of chromonyl chalcones, pyrazolines andtheir *in vitro* antibacterial, antifungal activities. *J. Enzyme Inhib. Med. Chem.*, 2012; *27:* 84-91.
13. Nowakowska Z. A review of anti-infective and anti-inflammatory chalcones. *Eur. J. Med. Chem.*, 2007; *42:* 125-137.
14. Yang HM, ShinH.-R, Cho S –H, Bang S –C, Song G –Y, Ju J.-H, Kim M.-K, Lee, S.-H, Ryu J.-C, Kim Y and Jung S.-H. Structural requirement of chalcones for the inhibitory activity of interleukin-5. *Bioorg. Med. Chem.*, 2007; *15:* 104-111.
15. Lee SH, Seo GS, Kim JY, Jin XY, Kim H-D and Sohn DH. Hemeoxygenase 1 mediates anti-inflammatory effects of 2,4,6-tris(methoxymethoxy) chalcone. *Eur. J. Pharmacol.*, 2006; *532*: 178-186.
16. Valla A, Valla B, Cartier D, Le Guillou R, Labia R, Florent L, Charneau S, Schrevel J and Potier P. New syntheses and potential antimalarial activities of new 'retinoid-like chalcones'. *Eur. J. Med. Chem.*, 2006; *41:* 142-146.
17. Domı-nguez JN, Charris JE, Lobo G, Gamboa de Dom, nguez N, MorenoMAM, Riggione F, Sanchez E, Olson J and Rosenthal PJ. Synthesis of quinolinyl chalcones and evaluation of their antimalarial activity. *Eur. J.Med. Chem.*, 2001; *36:* 555-560.
18. Liu M, Wilairat P and Go M-L. Antimalarial alkoxylated and hydroxylated chalcones: structure□activity relationship analysis. *J. Med. Chem.*, 2001; *44:* 4443-4452.
19. Seo WD, Ryu YB, Curtis-Long MJ, Lee CW, Ryu HW, Jang KC and Park KH. Evaluation of anti-pigmentary effect of synthetic sulfonylamino chalcone. *Eur. J. Med. Chem.*, 2010; *45:* 2010-2017.
20. Tomar V, Bhattacharjee G, Kamaluddin and Ashok K. Synthesis and antimicrobialevaluation of new chalcones containing piperazine or 2,5-dichlorothiophene moiety. *Bioorg. Med. Chem.*, 2007; *17:* 5321-5324.

21. Nowakowska Z, K-dzia B and Schroeder G. Synthesis, physicochemicalproperties and antimicrobial evaluation of new (*E*)-chalcones. *Eur. J. Med. Chem.*, 2008; *43:* 707-713.
22. Trivedi JC, Bariwal JB, Upadhyay KD, Naliapara YT, Joshi SK, Pannecouque CC, De Clercq E and Shah AK. Improved and rapid synthesisof new coumarinyl chalcone derivatives and their antiviral activity. *Tetrahedron Lett.*, 2007; *48:* 8472-8474.
23. Lin Y.-M, Zhou Y, Flavin MT, Zhou L.-M, Nie W and Chen F-C. Chalcones and flavonoids as anti-tuberculosis agents. *Bioorg. Med. Chem.*, 2002; *10:* 2795-2802.
24. Gacche RN, Dhole NA, Kamble SG and Bandgar BP. In-vitro evaluation of selected chalcones for antioxidant activity. *J. Enzyme Inhib. Med. Chem.*, 2008; *23:* 28-31.
25. Ducki S, Forrest R, Hadfield J A, Kendall A, Lawrence NJ, McGown AT and Rennison D. Potent antimitotic and cell growth inhibitory properties of substituted chalcones. *Bioorg. Med. Chem. Lett.*, 1998; *8:* 1051-1056.
26. Boeck P, Bandeira Falcão CA, Leal PC, Yunes RA, Filho VC, Torres-Santos EC and Rossi-Bergmann B. Synthesis of chalcone analogues with increased antileishmanial activity. *Bioorg. Med. Chem. Lett.*, 2006; *14:* 1538-1545.
27. Zhao L.-M, Jin H-S, Sun L-P, Piao H-Rand Quan Z-S. Synthesis and evaluation of antiplatelet activity of trihydroxychalcone derivatives. *Bioorg. Med. Chem. Lett.*, 2005; *15:* 5027-5029.
28. Bonesi M, Loizzo MR, Statti GA, Michel S, Tillequin Fand Menichini F. The synthesis and angiotensin converting enzyme (ACE) inhibitory activity of chalcones and their pyrazole derivatives. *Bioorg. Med. Chem. Lett.*, 2010; *20:* 1990-1993.
29. Dimmock JR, Elias DW, Beazely MA, Kandepu NM. Bioactivities of chalcones. *Curr. Med. Chem.*, 1999; *6:* 1125-1149.
30. Sahu NK, Balbhadra SS, Choudhary J, Kohli DV. Exploring pharmacologicalsignificance of chalcone scaffold: a review. *Curr. Med. Chem.*, 2012; *19:* 209-225.
31. Dhar, D.N. *The Chemistry of Chalcones and Related Compounds*, Wiley, New York, 1981.
32. Srinivasa Rao M, Kotesh J, Narukulla R and Duddeck H. Synthesis and spectroscopic characterization of some chromanochalcones and their dihydro derivatives, Arkivoc, 2004; xiv: 96-102.
33. Rajendra Prasad Y, lakshmana Rao A, Rambabu R and Ravi Kumar P. Synthesis and biologicaevaluation of some novel chalcone derivatives, Oriental J. Chem., 2007; 23(3): 927-937.

34. Fekria M A Soliman, Nadia T A Dawoud and Rehab M Hamza. Synthesis, Biological and Anti-tumor Evaluation of Some New Nucleosides Incorporating Heterocyclic Moieties. Med chem., 2015; 5:11, 496-504.
35. Yen GC, and Duh, PD. Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen species. J. Agric. Food Chem., 1994; 42(3): 629-632.
36. Lee K, Cho M, Miaskowski C, Dodd M. Impaired sleep and rhythms in persons with cancer. *Sleep Med Rev.*, 2004; 8: 199-212.
37. Ames BN, Gold LS. Dietary carcinogens, environmental pollution, and cancer: some misconceptions. *Med Oncol Tumor Pharmacother*, 1990; 7: 69-85.
38. WHO. 2015. Available online: http://www.who.int/ mediacentre/factsheets/fs297/en/
39. Lin S, Hoffmann K, Schemmer P. Treatment of hepatocellular carcinoma: A systematic review. Liver Cancer., 2012; 1: 144–158. doi: 10.1159/000343828.
40. Mazzanti R, Arena U, Tassi R. Hepatocellular carcinoma: Where are we? World J Exp Med., 2016; 6: 21–36. doi: 10.5493/wjem.v6.i1.21.
41. R. Dhanasekaran, A. Limaye, R.Cabrera, Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis, and therapeutics. Hepat Med., 2012; 4: 19–37.
42. S. Buschauer, A. Koch, P. Wiggermann, M. Müller And C. Hellerbrand, Hepatocellular carcinoma cells surviving doxorubicin treatment exhibit increased migratory potential and resistance to doxorubicin re-treatment *in vitro.* Oncol Lett., 2018 Apr; 15(4): 4635–4640.
43. Doxorubicin, Wikipedia, https://en.wikipedia.org/wiki/Doxorubicin#cite_note-AHFS2017-3
44. Tacar, O; Sriamornsak, P; Dass, CR (February 2013). "Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems". *The Journal of Pharmacy and Pharmacology*, 65(2): 157–70.
45. M. Suffness, J.M. Pezzuto, Methods in Plant Biochemistry, vol. VI, Academic press, New York, 1991; 71.
46. Gatti G, Lucini V, Dugnani S, Calastretti A, Spadoni G, Bedini A, Rivara S, Mor M, Canti G, Scaglione F2, Bevilacqua A1. Antiproliferative and pro-apoptotic activity of melatonin analogues on melanoma and breast cancer cells. Oncotarget., 2017 Aug 10; 8(40): 68338-68353.
47. Blask DE, Lemus-Wilson AM, Wilson ST. Breast cancer: a model system for studying the neuroendocrine role of pineal melatonin in oncology. *Biochem Soc Trans.*, 1992; 20: 309-311.

48. Hill SM and Blask DE: Effects of the pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. Cancer Res., 1988; 48: 6121-6126.
49. Cos S and Blask DE: Effects of the pineal hormone melatonin on the anchorage-independent growth of human breast cancer cells (MCF-7) in a clonogenic culture system. Cancer Lett., 1990; 50: 115-119.
50. Cos S and Sanchez-Barcelo EJ: Melatonin inhibition of MCF-7 human breast cancer cells growth: influence of cell proliferation rate. Cancer Lett., 1995; 93: 207-212.
51. Cos S, Ferna´ndez F, Sa´nchez-Barcelo´ EJ. Melatonin inhibits DNA synthesis in MCF-7 human breast cancer cells in vitro. Life Sci., 1996; 58: 2447–2453.
52. Wang JS, Xiao XS, Zhang Y, Shi DB, Chen WB, Fu LY, Liu LQ, Xie FY, Kang TB, Huang WL, Deng WG. Simultaneous modulation of COX-2, p300, Akt, and Apaf- 1 signaling by melatonin to inhibit proliferation and induce apoptosis in breast cancer cells. J Pineal Res., 2012; 53: 77- 90.
53. Mao LL, Yuan L, Slakey LM, Jones FE, Burow ME, Hill SM. Inhibition of breast cancer cell invasion by melatonin is mediated through regulation of the p38 mitogen-activated protein kinase signaling pathway. Breast Cancer Res., 2010; 12: R107.
54. Goncalves ND, Colombo J, Lopes JR, Gelaleti GB, Moschetta MG, Sonehara NM, Hellmen E, Zanon CD, Oliani SM, Zuccari D. Effect of melatonin in epithelial mesenchymal transition markers and invasive properties of breast cancer stem cells of canine and human cell lines. PloS One., 2016; 11: e0150407.
55. R. M. Moretti, M. Montagnani Marelli, R. Maggi, D. Dondi, Ma. Motta and P. Limonta. Antiproliferative action of melatonin on human prostate cancer LNCaP cells. Oncology Reports, 2000; 7: 347-351.
56. Si C. Xi, Stephanie W.F. Siu, Sze W. Fong, and Stephen Y.W. Shiu, Inhibition of Androgen-Sensitive LNCaP Prostate Cancer Growth In Vivo by Melatonin: Association of Antiproliferative Action of the Pineal Hormone Withmt1Receptor Protein Expression. The Prostate, 2001; 46: 52-61.
57. Marelli MM, Limonta P, Maggi R, Motta M, Moretti RM. Growth-inhibitory activity of melatonin on human androgen-independent DU 145 prostate cancer cells. Prostate, 2000; 45: 238–244.
58. Shiu SY. Towards rational and evidence-based use of melatonin in prostate cancer prevention and treatment. J Pineal Res., 2007; 43: 1–9.

59. J. Cabrera, G. Negrín, F. Estévez, J. Loro, R. J. Reiter and J. Quintana, Melatonin decreases cell proliferation and induces melanogenesis in human melanoma SK-MEL-1 cells. J. Pineal Res., 2010; 49: 45–54.
60. J. Perdomo, J. Cabrera, F. Estevez, J. Loro, R. J. Reiter and J Quintana, Melatonin induces apoptosis through a caspase-dependent but reactive oxygen species-independent mechanism in human leukemia Molt-3 cells. J. Pineal Res., 2013.
61. Farriol M, Venereo Y, Orta X, Castellanos JM, Segovia-Silvestre T. In vitro effects of melatonin on cell proliferation in a colon adenocarcinoma line. J Appl Toxicol, 2000; 20: 21–24.
62. M. Fic, A. Gomulkiewicz, J. Grzegrzolka, M. Podhorska-Okolow, M. Zabel, P. Dziegiel and K. Jablonska. The Impact of Melatonin on Colon Cancer Cells' Resistance to Doxorubicin in an In Vitro Study. *Int. J. Mol. Sci.*, 2017; *18*(7): 1396.
63. García-Navarro A, González-Puga C, Escames G et al. Cellular mechanisms involved in the melatonin inhibition of HT-29 human colon cancer cell proliferation in culture. J Pineal Res., 2007; 43: 195–205.
64. Petranka J, Baldwin W, Biermann J, Jayadev S, Barrett JC, Murphy E. The oncostatic action of melatonin in an ovarian carcinoma cell line. J Pineal Res., 1999; 26: 129–136.
65. García-Santos G, Antolín I, Herrera F et al. Melatonin induces apoptosis in human neuroblastoma cancer cells. J Pineal Res., 2006; 41: 130–135.
66. F. Long, C. Dong, K. Jiang, Y. Xu, X. Chi, D. Sun, R. Liang, Z Gao, S. Shao and L. Wang. Melatonin enhances the anti-tumor effect of sorafenib via AKT/p27-mediated cell cycle arrest in hepatocarcinoma cell lines. RSC Adv., 2017; 7: 21342 (The Royal Society of Chemistry).
67. J. Martín-Renedo, J. L. Mauriz, F. Jorquera, O. Ruiz-Andrés, P. González and J. González-Gallego, Melatonin induces cell cycle arrest and apoptosis in hepatocarcinoma HepG2 cell line. J. Pineal Res., 2008; 45: 532–540.
68. Zlotos, D.P.; Jockers, R.; Cecon, E.; Rivara, S.; Witt-Enderby, P.A. MT1 and MT2 Melatonin Receptors: Ligands, Models, Oligomers, and Therapeutic Potential. J. Med. Chem., 2014; 57: 3161–3185.
69. Anisimov VN, Popovich IG, Zabezhinski MA, Anisimov SV, Vesnushkin GM, Vinogradova IA. Melatonin: antioxidant, genoprotector and anticarcinogen. Biochim Biophys Acta, 2006; 1757: 573–589.
70. S. Carbajo-Pescador, A García-Palomo, J. Martín- Renedo, M. Piva, J. González-Gallego and J. L. Mauriz, Melatonin modulation of intracellular signaling pathways in

hepatocarcinoma HepG2 cell line: role of the MT1 receptor. J. Pineal Res., 2011; 51: 463–471.

71. S. Carbajo-Pescador, C Steinmetz, A Kashyap, S Lorenz, J L Mauriz, M Heise, P R Galle, J González-Gallego and S Strand, Melatonin induces transcriptional regulation of Bim by FoxO3a in HepG2 cells. British Journal of Cancer, 2013; 108: 442–449.
72. Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65: 55-63.