Research article

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Cytotoxicity, Anti-microbial activity and molecular docking simulation of novel Bis-chalcones linked to tetrahydro-[1,2,4]triazolo[3,4-*a*] isoquinoline moiety

Nada S. Ibrahim[a*], Elshimaa M. Eid[b], Huwaida M. E. Hassaneen[b], Hamdi M. Hassaneen[b], Ahmed H. M. Elwahy[b]*, Ismail A. Abdelhamid[b]*

[a]Chemistry Department (biochemistry branch), Faculty of Science, Cairo University, Giza, Egypt

[b]Chemistry Department, Faculty of Science, Cairo University, Giza-Egypt

Email: nasasabry@gmail.com, aelwahy@hotmail.com, ismail_shafy@yahoo.com

Abstract

Novel series of bis(tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)-3-arylprop-2-en-1-one) derivatives were prepared and their structures were confirmed by several spectral tools. Chalcone 11 was selected for our studies as an example of the series. Molecular docking studies revealed that compound 11 interacted with the active site of two members of inhibitor apoptotic proteins, XIAP-BIR3 and cIAP1- BIR3 with good energy score. Compund 11 showed three types of interactions with the active site of XIAP-BIR3.While compound 11 interacted with cIAP1-BIR3 through four modes of binding. Cytotoxicity test was done on colon carcinoma cell line (HCT116) and hepatic carcinoma cell line (HepG2). Compound 15 showed moderate activity against HCT116 at 100 and 50µg/ml with % inhibition 68.4% and 67.45% respectively. Compound 11showed percent of cytotoxic inhibition of 75% and 71% for HCT116 and HEPG2 respectively, at 100 µg/ml. Anti-microbial activity showed that compounds 8 and 11 displayed moderate activity against *S. aureus* with inhibition zones 12.3 and 15.6 mm, respectively, comparing to ampicillin positive control (22 mm).

Keywords: HepG2; IAPs; HCT116; *S. aureus*.

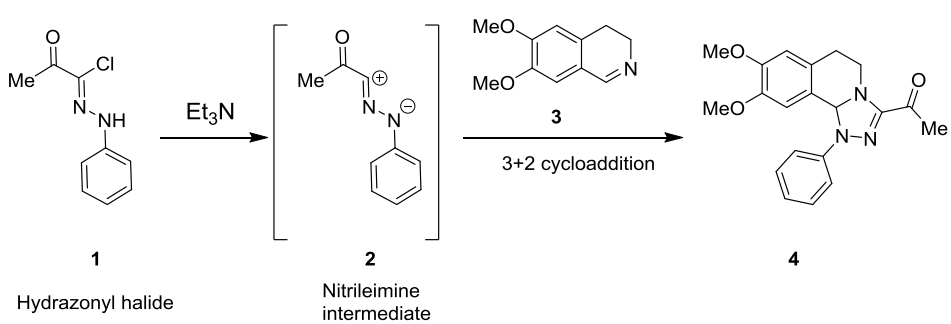
1. Introduction

Chalcone is a very interesting substrate that can be converted to flavanone by chalcone isomerase. Flavanone is the precursor for a variety of flavonoids which represent a class of plant-specific secondary metabolites that have various functions during plant growth and development. Moreover, chalcones exhibit various biological properties that include anticancer,[1–4] antiviral,[5] antiplatelet,[6] antibacterial,[2,7] anti-inflammatory,[8–10] analgesic,[11] antimalarial,[12] and antioxidant activities.[1,9] It is assumed that the presence of *a*, *ß*-unsaturated ketone in chalcones is considered to be the main reason of their bioactivity. In addition, heterocycles containing nitrogen such as [l,2,4]triazolo[3,4-*a*]isoquinolines showed remarkable pharmaceutical activities[13] such as cardiovascular,[13] antidepressant[13] and anti-inflammatory.[13] Moreover, several reports indicated that bis-heterocyclic compounds are amongst the bioactive heterocycles.[14–17] In continuation to our interest in synthesizing bioactive molecules[2,3,18–25] as well as bis(heterocyclic) compounds[21,26–34] we report herein the synthesis of novel bis(chalcones) incorporating [l,2,4]triazolo[3,4-*a*]isoquinoline ring systems. The cytotoxic as well as the antimicrobial activities of the novel compounds were also investigated.

2. Results and Discussion

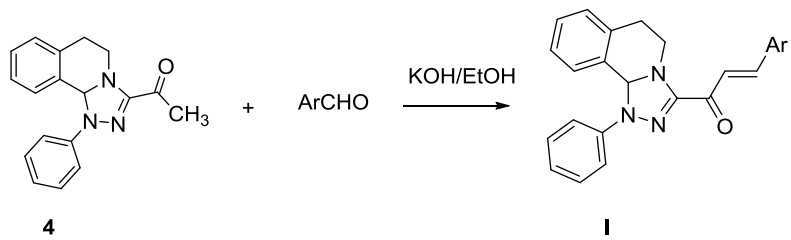
2.1. Synthetic Chemistry

The starting 3-acetyl-8,9-dimethyl-1-phenyl-1,5,6,10b-tetrahydro[1,2,4]triazolo[3,4-*a*]isoquinoline **4** was prepared with high yields following the reported procedures *via* the reactions of 3,4-dihydro-6,7-dimethoxyisoquinoline **3** with nitrilimines **2** that was obtained *in situ* upon treatment of the hydrazonyl chloride **1** with triethyamine (Scheme 1).[13,35]



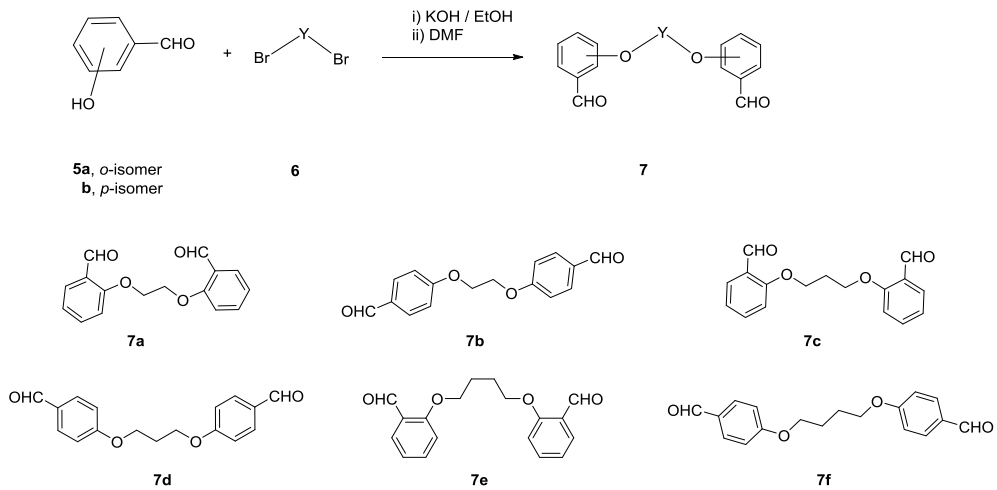
Scheme 1: Synthesis of 3-acetyl-tetrahydro-[1,2,4]triazolo[3,4-*a*]isoquinoline **4**

Recently, we reported the synthesis of some tetrahydro-[1,2,4]triazolo[3,4-*a*]isoquinolin-3-yl)-3-arylprop-2-en-1-one derivatives **I** by Claisen–Schmidt condensation of 3-acetyl-8,9-dimethoxy-1-phenyl-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-*a*]isoquinoline **4** with equimolar amounts of substituted aldehydes in the presence of potassium hydroxide solution (Scheme 2).[22] Cytotoxic evaluation of these compounds against different cancer cell lines (MCF7, A549, HCT116, and Hepg2) revealed that some of these compounds exhibited strong to moderate effect, while others showed weak action.[22]



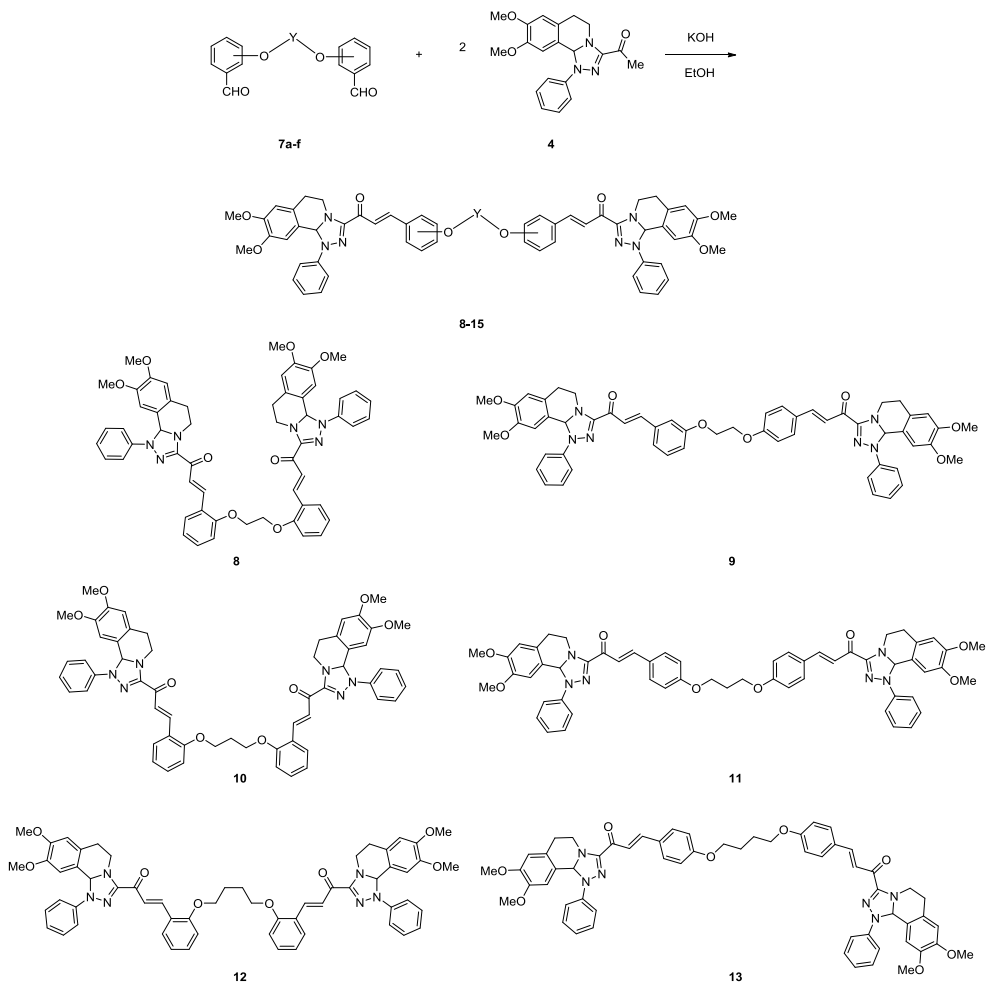
Scheme 2: The structure of Chalcones **I**

In an extension to this work, the bis(aldehydes) are used in a trial to affect condensation from both sides. The starting bis(aldehydes) **7a-f** were prepared as reported by our group, through the reaction of the potassium salt of the appropriate hydroxyaldehydes **5a** and **5b** with the corresponding dibromo compounds **6** in boiling DMF (Scheme 3).[36–42]



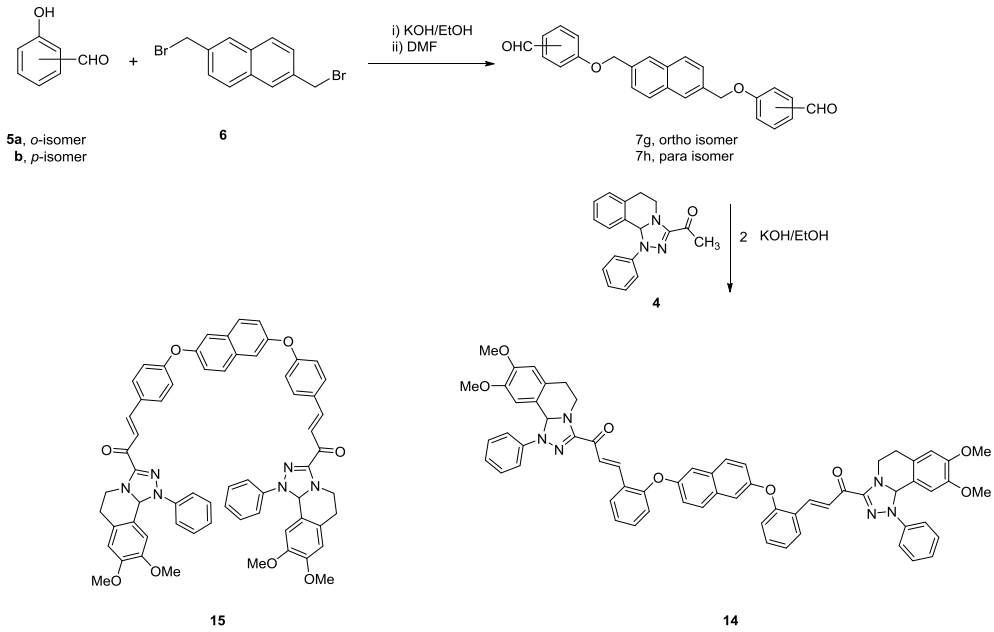
Scheme 3: Synthesis of the bis(aldehydes) **7a-f**

Claisen–Schmidt condensation of bis(aldehydes) **7a-f** with two equivalents of [1,2,4]triazolo[3,4-*a*]isoquinoline **4** in the presence of potassium hydroxide solution results in the formation of the corresponding bis(chalcone) derivatives **5-13** (Scheme 4). The structures of the formed products were elucidated by inspection of their spectral data.



Scheme 4: Synthesis of bis(tetrahydro-[1,2,4]triazolo[3,4-*a*]isoquinolin-3-yl)-3-arylprop-2-en-1-one) (**8-13**)

Bis-chalcones incorporated [1,2,4]triazolo[3,4-a]isoquinolines and liked together *via* naphthalene spacer were similarly prepared starting from bis-aldehyde **7g** and **7h** upon treatment with two equivalents of [1,2,4]triazolo[3,4-*a*]isoquinoline **4** in the presence of potassium hydroxide solution**.** The bis(aldehydes) **7g** and **7h** were prepared as previously reported by our group by the reaction of the potassium salt of the appropriate hydroxyaldehyde **5a** and **5b** with 2,6-bis(bromomethyl)naphthalene **6** in boiling DMF (Scheme 2).[36–42]



2.2. Bioactivity

2.2.1. Molecular docking:

The molecular docking studies were performed on two members of inhibitor apoptotic proteins family, X linked inhibitor apoptotic protein (XIAP-BIR3) and cellular inhibitor apoptotic protein 1 (cIAP1-BIR3) using Molecular operating environment (MOE) version 2009.10. Inhibitor apoptotic proteins (IAP) are members of a gene family that suppress apoptosis process against a lot of triggers and hence might contribute to the cancer development[295]. IAPs are structurally characterized by the presence of highly conserved baculoviral inhibitory repeat (BIR) domains[44], which are responsible for the inhibition of caspases especially the effector caspases -3 and -7.[45] Chalcone **11** was chosen as an example of the series. As shown in Fig. **1**, chalcone **11** interacted with the active site of XIAP-BIR3 with binding score equaled (-28.85 Kcal/mole) and three bindings, two arene-cation interactions, one was between benzene ring and Lys297 and the other was between benzene ring and Lys299. The third interaction was hydrogen bond between oxygen of methoxy group and Arg258 with bond distance 2.71 A^o. On the other hand, four interactions were recorded for chalcone **11** with cIAP1-BIR3 active site (Fig. **2**). The first one was hydrogen bond between oxygen and Lys322 with bond distance 2.47 A^o. The second was cation- cation interaction between benzene ring and Trp323. Finally, two hydrogen bonds were recorded between two different oxygens of methoxy group and Arg326 with bond distance

2.54 Aº and 2.39 Aº. It was found that the binding energy of the prepared compound was -28.8 Kcal/ mole.

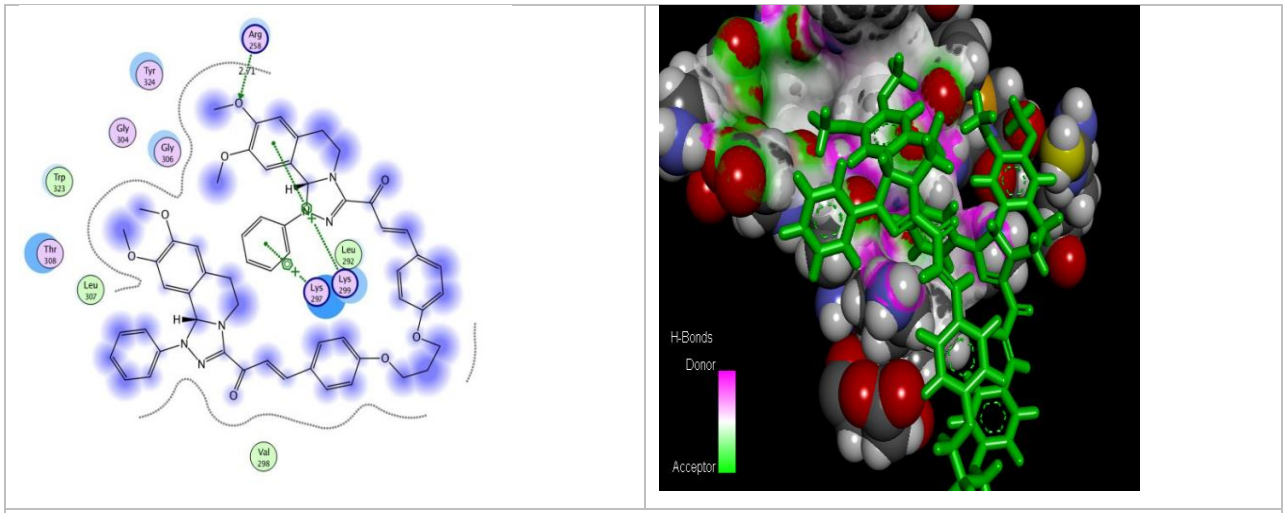


Figure 1: Chemical interaction of compound **11** with the active site of XIAP-BIR3 in 2D and 3D fashions.

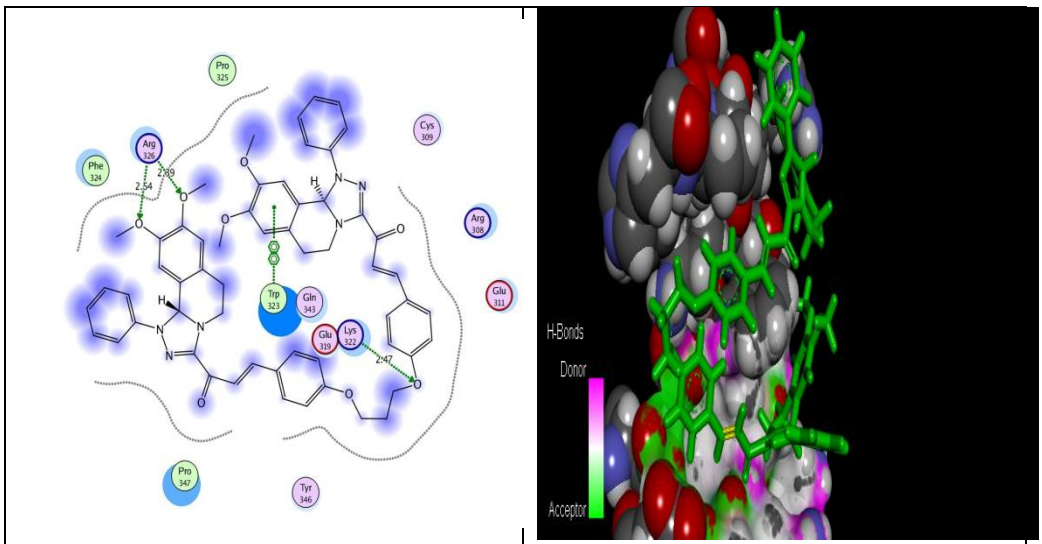


Figure 2: Chemical interaction of compound **11** with the active site of cIAP-BIR3 in 2D and 3D fashions.

2.2.2. Cytotoxic MTT assay:

In the present study, cytotoxicity was performed on a novel series of bis(tetrahydro-[1,2,4]triazolo[3,4-*a*]isoquinolin-3-yl)-3-arylprop-2-en-1-one) derivatives **8-12** and **15.** The data

obtained in Table **1** indicated that all compounds exhibited moderate to weak effect against all selected cancer cell lines. Chalcones derivatives were assayed against colon carcinoma (HCT116) and liver carcinoma (HEPG2) at concentrations 100 and 50 µg/ml. As demonstrated in Table **1**, four of the studied chalcone derivatives **8-10** and **12** showed no activity against both studied cancer cell lines, as the percent inhibition was lower than 50% at both concentrations (100 and 50 µg/ml). Among the prepared chalcones, chalcone **11** exerted cytotoxic activity against HCT116 and HEPG2 by inhibiting the growth of 75% and 71% of cancer cells, respectively, at 100 µg/ml. It was found that chalcone **15** showed moderate activity only against HCT116 by applying % inhibition of 68.4% and 67.45% at 100 and 50 µg/ml, respectively. It was reported from the previous studies that the mono derivatives of [1,2,4]triazolo[3,4-*a*]isoquinoline chalcones exhibited a promising cytotoxic activity against MCF7, HCT116, A549, and HepG2 cell lines.[22]

Table 1: % inhibition of the synthesized chalcones against HCT116 and HepG2 cancer cell lines at 100 and 50 µg/ml.

Compd No.	HCT116		HEPG2	
	% inhibition (100 µg/ml)	%inhibition(50 µg/ml)	%inhibition(100 µg/ml)	**% inhibition (50 µg/ml)**
8	43.49	25.65	44.13	**33.15**
9	11.6	10.2	24.5	**0**
10	34.12	0	32.46	**7.9**
11	75	33.9	71	**12**
12	27.2	25.2	48	**13.01**
15	68.4	67.45	32.67	**29.1**
Doxorubicin IC_{50} (µM)	2.2± 3.1		**0.6± 0.1**	

2.2.3. Antimicrobial assay:

Our synthesized chalcones were evaluated against gram negative bacteria (*E.coli* and *Klebsiella pneumonia*), gram positive bacteria (*S. aureus*) and fungal strain (*C. albicans*). All compounds exerted no activity against *E.coli* except compound **8** which showed little activity with inhibition

zone (9 mm) as compared to positive control, gentamicin (27 mm). All compounds showed no activity against *Klebsiella pneumonia* and *C. albicans*. On the other hand, chalcones **8** and **11** displayed moderate activity against *S. aureus* with inhibition zones (12.3 and 15.6 mm), respectively, comparing to ampicillin (22 mm) which was used as a positive control.

Table 2: Anti-microbial activity of the prepared chalcones in mm± SD.

	Gram negative bacteria (mm± SD)		Gram positive bacteria (mm± SD)	Fungi (mm± SD)
Compd	*Escherichia coli*	*Klebsiella pneumonia*	*Staphylococcus aureus*	*Candida albicans*
8	9± 0.1	NA	12.3± 0.5	NA
9	NA	NA	NA	NA
10	NA	NA	NA	NA
11	NA	NA	15.6± 0.5	NA
12	NA	NA	NA	NA
13	NA	NA	NA	NA
14	NA	NA	NA	NA
15	NA	NA	NA	NA
Ampicillin	--	--	22± 0.1	--
Gentamicin	27± 0.5	25± 0.5	--	--
Amphotericin B	--	--	--	21± 0.5

NA: indicated non active.

Conclusion

An efficient synthesis of bis-chalcones incorporating [l,2,4]triazolo[3,4-*a*]isoquinoline moiety has been carried out and their MTT cytotoxic activity was done on chalcones **8**- **12** and **15**. Chalcone **11** inhibited the proliferation of HCT116 with a percent of 68.4 and 67.45 at 100 and 50µg/ml respectively. The remaining compounds exerted no cytotoxic activity on both studied cancer cell lines HCT116 and HepG2. Anti-microbial activity was studied against gram negative bacteria (*E.coli* and *Klebsiella pneumonia*), gram positive bacteria (*S. aureus*) and fungal strain (*C. albicans*). Chalcones **8** and **11** showed moderate activity against *S. aureus.* The other compounds exerted no activity against the studied strains. Theoretical studies were performed using MOE program. Three interactions were recorded between chalcone **11** and XIAP-BIR3 and four interactions with the active site of cIAP1-BIR3.

3. Experimental

3.1. Chemistry

Melting points were measured with a Stuart melting point apparatus and are uncorrected. The IR spectra were recorded using a FTIR Bruker–vector 22 spectrophotometer as KBr pellets. The ^{1}H NMR spectra were recorded in DMSO–d_6 as solvent on Varian Gemini NMR spectrometer at 400 MHz using TMS as internal standard. Chemical shifts are reported as δ values in ppm. Mass spectra were recorded with a Shimadzu GCMS–QP–1000 EX mass spectrometer in EI (70 eV) model. The elemental analyses were performed at the Micro analytical center, Cairo University.

Synthesis of the bis(1-(8,9-dimethoxy-1-phenyl-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)prop-2-en-1-ones) (8-15)

A mixture of the appropriate bis(aldehydes) **7a-h** (1 mmol) and [1,2,4]triazolo[3,4-a]isoquinolin-3-yl)ethan-1-one **4** (0.702 g, 2 mmol) in ethanol (20 ml) was kept at 0-5 ^{o}C for 30 min. and potassium hydroxide (20%, 5 mL) was then added. The reaction mixture was then stirred at room temperature for 6 h, and then poured over ice water containing HCl. The formed solid was then filtered, washed with cold water and dried. The crude product was crystallized from ethanol to give chalcones **8**-**15**.

3,3'-((Ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis(1-(8,9-dimethoxy-1-phenyl-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)prop-2-en-1-one) (8)

Red crystals, (85%), m.p. 130-132 ^{o}C; IR (cm^{-1}): 1631 (C=O) ^{1}H NMR (DMSO-d_6); δ 2.66-2.82 (m, 4H, H6), 3.41 (s, 6H, OCH_3), 3.69 (s, 6H, OCH_3), 3.84-3.87 (m, 2H, H5), 4.16-4.58 (m, 6H, H5+2OCH_2), 6.62 (s, 2H, H10b), 6.77 (s, 2H, H7), 6.82-7.33 (m, 24H, H10 + 2 vinyl-H + Ar-H). MS *m/z* (%):936 (100), 937 (61). Anal. Calcd $C_{56}H_{52}N_6O_8$: C, 71.78; H, 5.59; N, 8.97. Found: C, 71.65; H, 5.68; N, 8.58.

3,3'-((Ethane-1,2-diylbis(oxy))bis(4,1-phenylene))bis(1-(8,9-dimethoxy-1-phenyl-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)prop-2-en-1-one).(9)

Red crystals, (87%), m.p. 135-138 ^{o}C; IR (cm^{-1}): 1648 (C=O) ^{1}H NMR (DMSO-d_6); δ 2.66-2.84 (m, 4H, H6), 3.41 (s, 6H, OCH_3), 3.72 (s, 6H, OCH_3), 3.86-3.88 (m, 2H, H5), 4.20-4.41 (m, 6H, H5+2OCH_2), 6.66 (s, 2H, H10b), 6.78 (s, 2H, H7), 6.95-7.77 (m, 24H, H10 + 2 vinyl-H + Ar-H). ^{13}C NMR (DMSO-d_6), δ 27.3, 41.9, 55.7, 55.9, 67.0, 78.0, 109.3, 112.4, 115.3, 115.6, 120.7, 121.4, 127.6, 127.8, 128.8, 129.8, 131.2, 141.9, 143.8, 147.5, 149.0, 149.9, 160.8, 179.4.

MS *m/z* (%):936 (100), 937 (61). Anal. Calcd $C_{56}H_{52}N_6O_8$: C, 71.78; H, 5.59; N, 8.97. Found: C, 71.65; H, 5.69; N, 8.78.

3,3'-((Propane-1,3-diylbis(oxy))bis(2,1-phenylene))bis(1-(8,9-dimethoxy-1-phenyl-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)prop-2-en-1-one)) (10)

Red crystals, (90%), m.p. 140-143 ^oC; IR (cm^{-1}): 1642 (C=O). ^1H NMR (DMSO-d_6); δ 2.39-2.42 (m, 2H, CH_2), 2.64-2.79 (m, 4H, H6), 3.39 (s, 6H, OCH_3), 3.71 (s, 6H, OCH_3), 3.86-3.89 (m, 2H, H5), 4.19-4.25 (m, 2H, H5), 4.31 (t, 4H, CH2), 6.65 (s, 2H, H10b), 6.77 (s, 2H, H7), 6.94-7.89 (m, 24H, H10 + 2 vinyl-H + Ar-H). ^{13}C NMR (DMSO-d_6), δ 27.3, 29.1, 55.7, 55.9, 65.5, 78.1, 109.4, 112.5, 113.1, 115.2, 120.6, 121.5, 123.3, 123.4, 127.5, 128.8, 129.7, 130.0, 132.5, 137.1, 143.7, 147.5, 149.0, 149.9, 158.1, 179.7. MS *m/z* (%):950 (100), 951 (64). Anal. Calcd $C_{57}H_{54}N_6O_8$: C, 71.98; H, 5.72; N, 8.84. Found: C, 71.67; H, 5.84; N, 8.52.

3,3'-((Propane-1,3-diylbis(oxy))bis(4,1-phenylene))bis(1-(8,9-dimethoxy-1-phenyl-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)prop-2-en-1-one).(11)

Red crystals, (90%), m.p. 142-146 ^oC; IR (cm^{-1}): 1642 (C=O). ^1H NMR (DMSO-d_6); δ 2.22-2.23 (m, 2H, CH_2), 2.65-2.80 (m, 4H, H6), 3.42 (s, 6H, OCH_3), 3.72 (s, 6H, OCH_3), 3.79-3.90 (m, 2H, H5), 4.21-4.27 (m, 6H, H5 + $2OCH_2$), 6.66 (s, 2H, H10b), 6.78 (s, 2H, H7), 6.97-7.61 (m, 24H, H10 + 2 vinyl-H + Ar-H).^{13}C NMR (DMSO-d_6): δ 27.3, 28.8, 41.9, 55.7, 55.9, 65.2, 77.9, 109.4, 112.5, 115.2, 120.5, 121.4, 127.6, 128.8, 129.8, 131.1, 132.3, 141.9, 143.8, 147.5, 149.0, 150.0, 161.1, 163.9, 179.4. MS *m/z* (%): 950 (100), 951 (64). Anal. Calcd $C_{57}H_{54}N_6O_8$: C, 71.98; H, 5.72; N, 8.84. Found: C, 71.69; H, 5.54; N, 8.97.

3,3'-((Butane-1,4-diylbis(oxy))bis(2,1-phenylene))bis(1-(8,9-dimethoxy-1-phenyl-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)prop-2-en-1-one). (12)

Red crystals, (85%), m.p. 176-178 ^oC; IR (cm^{-1}): 1644 (C=O). ^1H NMR (DMSO-d_6); δ 2.06 (br, 4H, CH_2), 2.63-2.79 (m, 4H, H6), 3.39 (s, 6H, OCH_3), 3.70 (s, 6H, OCH_3), 3.79-3.89 (m, 2H, H5), 4.13-4.23 (m, 6H, H5+$2OCH_2$), 6.63 (s, 2H, H10b), 6.76 (s, 2H, H7), 6.92-7.88 (m, 24H, H10 + 2 vinyl-H + Ar-H). ^{13}C NMR (DMSO-d_6): δ 26.1, 27.3, 41.9, 55.7, 55.9, 68.3, 77.9, 109.3, 112.4, 112.9, 115.2, 121.2, 121.4, 123.2, 123.4, 127.5, 128.8, 129.7, 130.3, 132.5, 137.3, 143.8, 147.4, 149.0, 150.0, 158.3, 179.8. MS *m/z* (%): 964 (100), 965 (63). Anal. Calcd $C_{58}H_{56}N_6O_8$: C, 72.18; H, 5.85; N, 8.71 Found: C, 742.20; H, 5.83; N, 8.99.

3,3'-((Butane-1,4-diylbis(oxy))bis(4,1-phenylene))bis(1-(8,9-dimethoxy-1-phenyl-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)prop-2-en-1-one). (13)

Red crystals, (87%), m.p. 172-174 ^{o}C IR (cm^{-1}): 1644 (C=O). ^{1}H NMR (DMSO-d_6); δ 1.9 (br, 4H, CH_2), 2.69-2.79 (m, 4H, H6), 3.4 (s, 6H, OCH_3), 3.66 (s, 6H, OCH_3), 3.79-4.11 (m, 8H, H5 +2OCH_2), 6.66 (s, 2H, H10b), 6.77 (s, 2H, H7), 6.93-7.87 (m, 24H, H10 + 2 vinyl-H + Ar-H). MS *m/z* (%): 964 (100), 965 (62). Anal. Calcd $C_{58}H_{56}N_6O_8$: C, 72.18; H, 5.85; N, 8.71. Found: C, 72.25; H, 5.93; N, 8.83.

3,3'-(((Naphthalene-2,6-diylbis(methylene))bis(oxy))bis(2,1-phenylene))bis(1-(8,9-dimethoxy-1-phenyl-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)prop-2-en-1-one). (14)

Red crystals, (80%), m.p. 150 ^{o}C; IR (cm^{-1}): 1642 (C=O). ^{1}H NMR (DMSO-d_6); δ 2.60-2.79 (m, 4H, H6), 3.41 (s, 6H, OCH_3), 3.70 (s, 6H, OCH_3), 3.81-3.87 (m, 2H, H5), 4.15-4.28 (m, 2H, H5), 5.22-5.38 (m, 4H, OCH_2), 6.66 (s, 2H, H10b), 6.74 (s, 2H, H7), 6.92-8.11 (m, 30H, H10 + 2 vinyl-H + Ar-H). MS *m/z* (%):1062 (100), 1063 (73). Anal. Calcd $C_{66}H_{58}N_6O_8$: C, 74.56; H, 5.50; N, 7.90. Found: C, 74.69; H, 5.63; N, 7.77.

3,3'-(((Naphthalene-2,6-diylbis(methylene))bis(oxy))bis(4,1-phenylene))bis(1-(8,9-dimethoxy-1-phenyl-1,5,6,10b-tetrahydro-[1,2,4]triazolo[3,4-a]isoquinolin-3-yl)prop-2-en-1-one) (15)

Red crystals, m.p. 153 ^{o}C; IR (cm^{-1}): 1642 (C=O). ^{1}H NMR (DMSO-d_6); δ 2.62-2.78 (m, 4H, H6), 3.38 (s, 6H, OCH_3), 3.69 (s, 6H, OCH_3), 3.85-3.89 (m, 2H, H5), 4.18-4.22 (m, 2H, H5), 5.38 (s, 4H, OCH_2), 6.64 (s, 2H, H10b), 6.74 (s, 2H, H7), 6.90-8.03 (m, 30H, H10 + 2 vinyl-H + Ar-H). ^{13}C NMR (DMSO-d_6): δ 27.3, 41.9, 55.7, 55.9, 70.4, 78.1, 109.3, 112.4, 113.8, 115.2, 121.4, 121.7, 123.4, 123.7, 126.3, 126.6,127.5, 128.7, 128.8, 129.7, 132.6, 132.8, 135.0, 143.6, 147.4, 149.0, 150.0, 157.9, 179.7. MS *m/z* (%):1062 (100), 1063 (73). Anal. Calcd $C_{66}H_{58}N_6O_8$: C, 74.56; H, 5.50; N, 7.90. Found: C, 74.79; H, 5.63; N, 7.77.

3. 2. Biological assays

3. 2. 1. Molecular docking

All molecular modeling calculations and docking studies were done using ‘‘Molecular Operating Environment (MOE) version 2009.10 release of Chemical Computing Group’s’’. The program was subjected to ‘‘Windows XP’’ operating system installed on an Intel Pentium IV PC with 2.9 MHz processor and 512 RAM. The prepared chalcones were built using the MOE builder interface and subjected to energy minimization using the included MOPAC. The produced model was subjected to Systematic Conformational Search where all items were set as default with RMS gradient of 0.01 kcal/mole and RMS distance of 0.1 A^{o}. The X-ray crystallographic structure of the inhibitor apoptotic proteins (IAPs), cellular inhibitor apoptotic protein 1 (cIAP1-BIR3) and X-linked inhibitor apoptotic protein (XIAP-BIR3) complexed with their ligands (PDB ID: 4KMN and 3EYL), respectively, were obtained from the protein data bank. The proteins were prepared for docking studies where: A. The bound ligand molecule was removed from the protein active site. B. Hydrogen atoms were added to the isolated target with their standard geometry. C. MOE alpha site finder was used for specifying the active sites in the protein structure and then dummy atoms were created from the obtained alpha spheres. D. the obtained model was then used in predicting the target ligand-protein interactions at the active site. Three dimensional (3D) model of the interacted compound was determined using Discovery studio 2016 client program.

Cell culture

Colon (HCT-116) and liver (HepG2) cancer cell lines were purchased from American Tissue Culture Collection (Rockville, MD, USA). HCT-116 and HepG2 Human cancer cells were maintained in DMEM medium (Lonza Biowahittkar, Belgium). All the media were supplemented with 1% antibiotic-antimycotic mixture (10,000 µg ml-1 streptomycin sulfate, 10,000 U ml^{-1} potassium penicillin, 25 µg ml^{-1} amphotericin B and 1% L-glutamine (Biowest, USA).

3.2.2. MTT cytotoxicity assay

Cell viability was studied using MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (Bio Basic Canada Inc., Canada). All steps were done in sterile laminar air flow cabinet Biosafety class II level (Baker, SG403INT; Sanford, ME, USA). All incubations were carried out at 37 ^{o}C in 5% CO_2 incubator in the humidified atmosphere (Sheldon, TC2323; Cornelius, OR, USA). Cells were seeded into 96-well microtiter plastic plates at the

concentration of (10^4 cells per well) and left for 24 h to allow the adherence of cells. Medium was sucked and fresh medium (without serum) was added to the cells with 100 μg ml^{-1} in DMSO then the compounds under the test were added at 100 and 50 μg ml-1 in DMSO and incubated for 72 h. The medium was aspirated and 40 μl MTT salts (2.5μg ml-1) was added to each well and incubated for a further 4 h. 200 μl of 10 % sodium dodecyl sulfate (SDS) were added to each well and incubated overnight at 37 ºC in order to stop the reaction and dissolve any formed formazan crystals. The amount of formazan product was measured at wavelength of 595 nm with a reference wavelength of 620 nm using a microplate reader (Bio-Rad Laboratories, model 3350, USA). For the untreated cells (negative control), medium was added instead of the target compounds. A positive control Adrinamycin® (doxorubicin) was used as a known cytotoxic natural agent giving 100% inhibition. Dimethyl sulfoxide (DMSO) was used as a vehicle for dissolution of testing compound, and its final concentration on the cells was less than 0.2%. The statistical analysis was done by using Prism software program (Graph Pad software incorporated, version 3).

3. 2. 3. Antimicrobial assay

All the synthesized chalcones were tested *in vitro* for their antibacterial activity against *staphylococcus aureus* (ATCC: 6538) (Gram positive bacteria), *Escherichia coli* (ATCC: 9637) and *klebsiella pneumonia* (ATCC: 10031) (Gram negative bacteria) using nutrient agar medium. Ampicillin and Gentamicin were used as standard drugs for gram positive and gram negative respectively. Antifungal activity was done against *Candida albicans* (ATCC: 10231) using amphotericin B as a positive control. DMSO was used as solvent control. The compounds were tested at a concentration of 15 mg/ml against both bacterial and fungal strains. The antimicrobial activity of the target compounds was assayed using agar well diffusion method.[46] The sterilized media was poured onto the sterilized Petri dishes (20-25 ml, each petri dish) and allowed to be solidified at room temperature. Bacterial and fungal suspensions were prepared in sterilized saline equivalent to McFarland 0.5 standard solution ($1.5x\ 10^5$ CFU mL^{-1}) and its turbidity were adjusted to OD = 0.13 using spectrophotometer at 625 nm. Optimally, within 15 minutes after adjusting the turbidity of each inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension and was flooded on the dried nutrient agar surface then allowed to dry for 15 minutes with lid in place. Wells of 6 mm diameter was made in the

solidified media with the help of sterile borer. A solution of the target compound 100 μL of was added to each well using micropipette. The plates were incubated at 37 °C for 24 h. This experiment was carried out in triplicate to measure the standard deviation (SD) using SPSS statistical analysis software package/version 11.0. Zones of inhibition were measured in mm. scale.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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

Informed consent For this type of study, formal consent is not required.

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