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Research paper

Design, synthesis and cytotoxic activity of certain novel chalcone analogous compounds

S. El-Meligie [a], Azza T. Taher [a, b], Aliaa M. Kamal [a, *], A. Youssef [c]

[a] Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Cairo University, 11561, Cairo, Egypt
[b] Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza, Egypt
[c] Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy and Drug Manufacturing, Misr University for Science and Technology, Giza, Egypt

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ABSTRACT

A series of chalcone analogous compounds were designed and synthesized. Replacing/substituting the enone or ethylenic bridge of the parent chalcone with rigid heterocyclic moieties or substituted aromatic amines gave nineteen target compounds. Their cytotoxic activities were screened against both breast and liver cancer cells as well as breast and liver normal cells. Target compounds were also evaluated for their inhibition activity of tubulin beta polymerization. Target compound **2e, 3a, 3b, 3c, 4a-4d, 5a, 5b** and **6** showed broad spectrum excellent anticancer activity against both MCF-7 and HepG2. Compound **4a** showed the most TUBb inhibition activity.

1. Introduction

Cancer, the uncontrolled, rapid and pathological proliferation of abnormal cells, is one of the most formidable afflictions in the world. Targeted therapies that interfere with a single biological molecule or pathway have been successfully utilized in treating cancer [1,2]. Tubulin heterodimers, the major component of microtubules, are a molecular target of anticancer therapy based on microtubule-targeting agents. Inhibition of tubulin polymerization or interfering with microtubule assembly/disassembly disrupts several cellular functions. Tubulin targeting agents constitute an important class of anticancer drugs and it includes several agents, such as colchicine (**I**), combretastatin A-4 (**II**), MDL-27048 (**III**) (Fig. 1), *vinca* alkaloids and a number of structurally unrelated small molecules. Two different binding sites on tubulin are known, namely the colchicine and the *vinca* alkaloid binding sites [3–19].

Chalcones (a series of biaryl propenones) have important effects on cancer cell growth and proliferation. Many mechanisms of action have been identified, including the inhibition of tubulin assembly, inhibition of angiogenesis, induction of apoptosis, antiestrogenic activity and reversal of multidrug resistance – or a combination of these mechanisms. Chalcones were powerful tubulin assembly inhibitors, with almost similar potency to combretastatin A4 (**II**, CA-4). Chalcone and related compounds as MDL-27048, CA-4, chalcone epoxides, related dienones and piperazine nucleus containing novel chalcones have been reported to possess great cytotoxic activity [7–10,12,14,15,18]. Many structure activity relationship studies (SAR) and a number of ligand- and structure based docking studies have been reported concerning the tubulin targeting agents [6,20].

During the last decade, several chalcone analogs and derivatives have been designed with the enone moiety replaced by a heterocyclic ring in order to get rigid analogs [1].

Pyrimidines, pyrazoles and epoxides nuclei exhibited remarkable pharmacological activities. Literature indicates that compounds having pyrimidine nucleus have wide range of therapeutic uses that include anticancer activity [21–24].

Structure-activity relationship (SAR) studies indicated ring A and ring C are crucial for colchicine-tubulin binding, whereas ring B, although important, did not emerge as essential for the activity and was shown to be tolerant to structural modifications where the hydroxyl group was not essential while the 4-methoxyphenyl group seemed crucial for cytotoxicity [1,25].

Docking studies of enormous colchicine site inhibitors (CSI) showed that, 7-point pharmacophore connected the different

* Corresponding author.
E-mail addresses: Aliaa.kamal@pharma.cu.edu.eg, K_aliaa2511@hotmail.com (A.M. Kamal).

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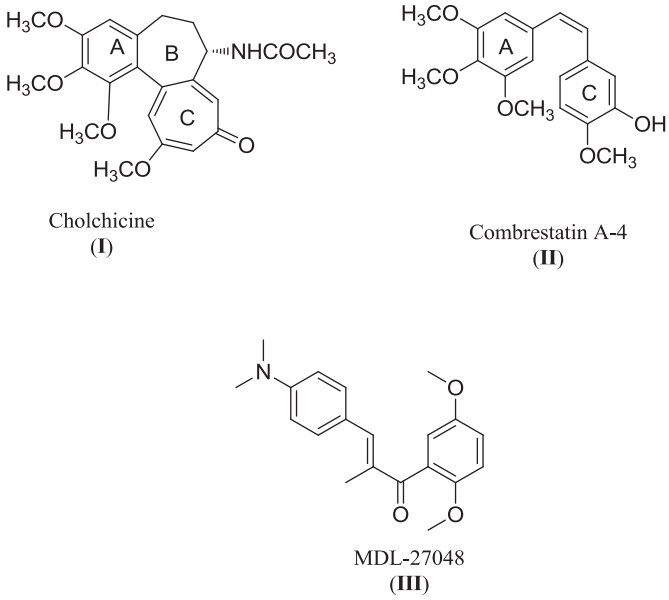


Fig. 1. Tubulin-targeting agents.

structural classes of CSI, based on consistent structural features and recurring tubulin–ligand interactions [1,5,6]. Three hydrogen bond acceptors, one hydrogen bond donor, two hydrophobic centers and one planar group were the 7-point pharmacophores. Hydrophobic centers and planar groups' points represented the rigid portion of the molecular scaffold, while the other features (the different heterocyclic of substituted amino alcohol bridge) represented critical interactions with the protein, thus being responsible for binding specificity. It should be noted that, none of the CSI drugs possessed all of the 7-points pharmacophore, suggesting that the discovery of more potent tubulin destabilizing agents could be a feasible task [1,5,6].

The target of this work was the development of novel compounds bearing chalcone moieties hybridized with epoxides, pyrimidines or pyrazoles via modification of the chalcones' structure involved either the enone (type 1)- or the alkene (type 2&3)-functionalized parts of parent chalcones. (Fig. 2).

All the synthesized compounds (Scheme 1) were characterized by two aromatic rings (A&C) linked by various fragments e.g. heterocyclic three, five or six membered rings and chalcone derivatives.

2. Results and discussion

2.1. Chemistry

In the present study, compounds (*E*)-3-(4-substituted phenyl)-1- n-(methoxyphenyl) prop-2-en-1-ones **(1a-i)** were obtained by the Claisen-Schmidt condensation of substituted acetophenones and benzaldehydes, affording exclusively the *E*-isomer in high yields [14]. Replacing the enone moiety with pyrimidine and hydroxypyrazole heterocycles took place by reacting the parent chalcones **1a-i** with thiourea to afford **2a-g** or reacting the epoxide derivatives of the parent chalcone **3a-d** with either phenyl hydrazine, hydrazine hydrate or thiourea to yield compounds **4a-f** and compound **A** respectively (Scheme 1). Upon using the conventional method for preparing compounds **2a-g**, it took very long time to complete the reaction with moderate yield. A novel highly efficient rapid method with very mild reaction conditions was used herein to synthesize compounds **2a-g** in high yield, high atom economy (less chemical wastes) and following environmental friendly protocol. Under microwave irradiation, the reaction time reduced to 3–4 h and the product was afforded in almost high yield. The progress of the reaction was monitored by TLC after every 1 h (Scheme 1).

The structure of the compounds **2a-g** was confirmed by element analysis, IR, ^{1}H-NMR, ^{13}CNMR and mass spectroscopy. The spectral data revealed the formation of the 3,4-dihydropyrimidinethione rather than the fully unsaturated pyrimidine. ^{1}H-NMR of compounds **2a-g** showed the appearance of two doublets corresponded to C4 proton due to geometrical isomerism and a doublet at δ 6.81–6.92 ppm due to C5 proton of the pyrimidine ring. ^{13}CNMR showed the characteristic C=S signal at δ 173.7–175.7 ppm and the characteristic signal of C4 at δ 60.4–60.6 ppm.

Replacement of the ethylenic bridge with oxirane ring via epoxidation of α,β–unsaturated ketones gave 3-aryl-2-substituted-acetophenoyl-oxiranes **3a-d**. Reacting compounds **1a-d** with hydrogen peroxide 28% in a mixture of polar solvents (methanol and acetone) at 0 °C afforded compounds **3a-d** in excellent yield. The structure of the products **3a-c** was confirmed by element analysis, IR, ^{1}HNMR, ^{13}CNMR and mass spectroscopy. IR spectra of **3a-c** showed an absorption band at 3446-3462 cm^{-1} indicating the presence of C–H of oxirane ring functionality. ^{1}HNMR spectra of **3a-c** showed two doublet signals at 4.13–4.17 ppm and δ 4.76–4.82 ppm corresponding to CH–O–CH of oxirane ring. Epoxides are versatile intermediate in organic synthesis as their ring can be easily opened by a variety of nucleophiles e.g. amines and hydrazines. To replace the enone bridge with hydroxypyrazole moiety; hydrazinolysis of the oxirane derivatives **3a-d** using either phenyl hydrazine or hydrazine hydrates was carried out to give compounds 5-aryl-N^{1}-substituted-3-(substituted-methoxyphenyl)-4-hydroxypyrazolines **4a-f** in good yield. The reaction of the hydrazine derivatives and epoxy chalcone analogues was believed to proceed via the formation of the intermediate hydrazone. IR, ^{1}HNMR, ^{13}CNMR and mass spectroscopy and element

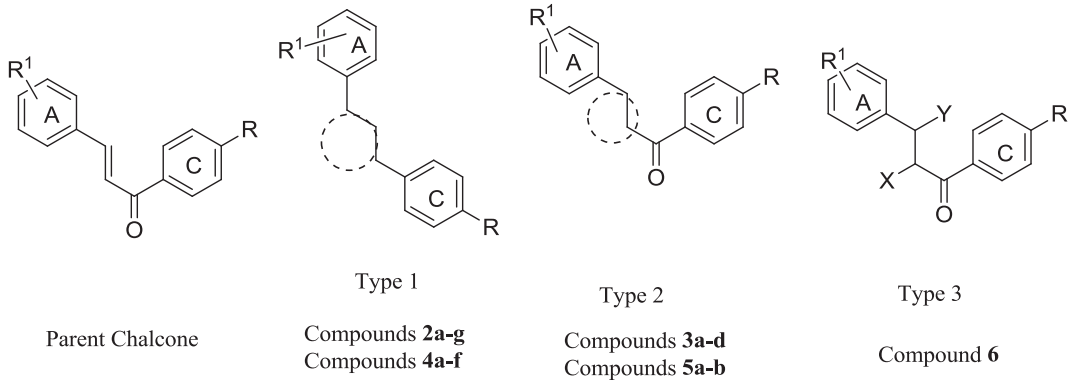
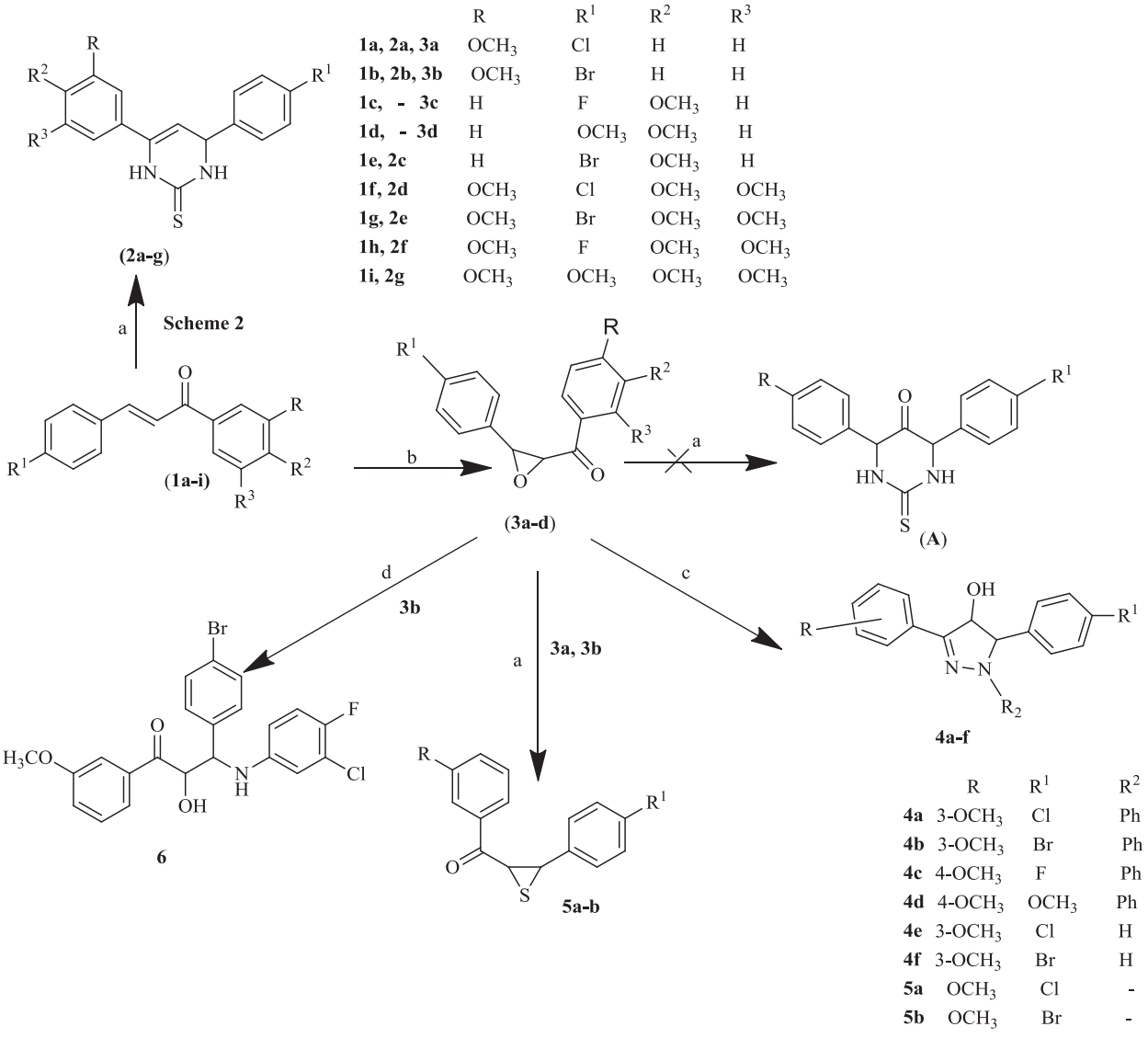


Fig. 2. General structural formulae of the target compounds.



	R	R^1	R^2	R^3
1a, 2a, 3a	OCH_3	Cl	H	H
1b, 2b, 3b	OCH_3	Br	H	H
1c, - 3c	H	F	OCH_3	H
1d, - 3d	H	OCH_3	OCH_3	H
1e, 2c	H	Br	OCH_3	H
1f, 2d	OCH_3	Cl	OCH_3	OCH_3
1g, 2e	OCH_3	Br	OCH_3	OCH_3
1h, 2f	OCH_3	F	OCH_3	OCH_3
1i, 2g	OCH_3	OCH_3	OCH_3	OCH_3

	R	R^1	R^2
4a	3-OCH_3	Cl	Ph
4b	3-OCH_3	Br	Ph
4c	4-OCH_3	F	Ph
4d	4-OCH_3	OCH_3	Ph
4e	3-OCH_3	Cl	H
4f	3-OCH_3	Br	H
5a	OCH_3	Cl	-
5b	OCH_3	Br	-

Reagents and condiitons: a) NH_2CSNH_2, NaOH; b) H_2O_2 / NaOH, c) Phenyl hydrazine / hydrazine hydrate; d)primary amine;

Scheme 1. Synthesis of compounds **2a-g, 3a-d, 4a-f, 5a-b** and **6.**

analysis were used to identify compounds **4a-f.** The ^{1}HNMR spectra of **4a-f** showed signals corresponding to C4 proton, C5 proton of pyrazole ring and the hydroxyl group. More over and as a result of the geometrical isomerism, two adjacent singlet signals of the methoxy methyl group were appeared.

Replacing the Enone Bridge with fully saturated pyrimidinone thione compound **A** was believed to be via reacting the oxirane derivatives of chalcone with thiourea, but upon carrying out this reaction a different product was formed. The spectral data of the obtained products proved the formation of thiirane derivatives of the chalcone **5a-b** rather than compound **A**. ^{1}HNMR spectra the **5a-b** should two doublet signals due to C2 and C3 protons of the thiirane ring at δ 4.15–4.17, 4.81–4.82 ppm. ^{13}CNMR spectrum of compound **5a** showed the characteristic signals of C2 and C3 protons of the thiirane ring at δ 38.9 and 59.7 ppm. Aminolysis of the epoxide chalcones by amines resulted in β-amino alcohols, which are important intermediates in the synthesis of a large number of biologically active natural and synthetic products. To replace the ethylenic bridge with substituted amine, compound **3b** was reacted with substituted aniline to give 3-(4-bromophenyl)-3-(3-chloro-4-fluorophenylamino)-2-hydroxy-1-(3-methoxyphenyl) propan-1-one (**6**) in very good yield. ^{1}HNMR of compound **6** showed four doublet signals at δ 4.78, 5.04, 5.84 and 6.36 ppm corresponds to CH–N, CH–O, OH and NH respectively.

2.2. Discussion of the cytotoxic activity of the target compounds

All the test compounds were evaluated for their cytotoxic activity against MCF-7 and HepG2 cell lines. They showed excellent cytotoxic activity, much more potent than or even as good as the reference drug colchicine.

2.2.1. Activity against the breast cancer cell line (MCF-7)

In the present study, we synthesized nineteen novel compounds. They were tested for their cytotoxic activity against MCF-7 cell line using colchicine as a reference drug.

Replacement of Enone Bridge of compounds **1a-i** with 3,4-dihydropyrimidine-2-thione moiety (Type 1) represented compounds **2a-g** which showed cytotoxic activity as potent as or more potent than the reference drug. Compounds **2d-g** (with trimethoxyphenyl (TMP) moiety (ring A)) showed higher cytotoxic activity against MCF-7 cell line than **2a-c**. Compound **2e** (IC_{50} 0.0770 μM) with TMP (ring A) and bromophenyl (ring C) was the most active amongst compounds **2a-g** nearly double the potency of the reference drug (IC_{50} 0.1504 μM).

Replacing the ethylenic bridge with oxirane resulted in compounds **3a-d** (type 2). Compounds **3a-c** showed excellent cytotoxic activity against MCF-7 much more potent than colchicine. The most active amongst this group were **3b** and **3c** with IC_{50} 0.0130 μM and IC_{50} 0.0140 μM respectively, about ten folds more potent than colchicine (IC_{50} 0.1504 μM). It was noticed that changing of the halogen group (ring C) from Br/F (compounds **3b** and **3c**) to Cl (compound **3a**) reduced the potency nearly to the half.

Replacing the Enone Bridge by hydroxypyrazole afforded compounds **4a-f** (type 1) which were far more potent than the reference drug. Compounds **4a-d** with N1-phenyl moiety revealed excellent antiproliferative activity against MCF-7 cell line (ten folds more potent), while **4e** and **4f** showed moderate activity in comparison to compound **4a**, the parent of this group. In fact, compounds **4a** and **4b** were amongst the most active ones against MCF-7 of all the test compounds.

Upon replacing the ethylenic bridge with thiirane heterocycle, compounds **5a** and **5b** resulted (type 2). The activity of compound **5a** against MCF-7 was one and half the cytotoxic activity of compound **5b.**

Substituting the ethylenic bridge with diphenyl ethanolamine moiety (type 3) resulted in one of the most potent test compounds **6** (Table 1).

2.2.2. Activity against human liver cancer cell line (HepG2)

Generally, all the novel synthesized compounds showed more potent cytotoxic activity against human liver carcinoma cell line (HepG2) than MCF-7 cell line. Replacing the enone bridge (Type 1) or the ethylenic bridge (Type 2&3) with either 3,4-dihydropyrimidine-2-thione, oxirane, pyrazoles, thiirane or diphenyl ethanolamine afforded compounds **2a-g, 3a-d, 4a-f, 5a-b** and **6** respectively.

In spite of being moderately active against MCF-7; compounds **2c, 2d,** and **2e** were the most active of this group (**2a-g**) with IC_{50} (0.0300, 0.0110 and 0.0095 μM) respectively against human hepatic carcinoma cell line (HepG2). Moreover, Compounds **3a** and **3c** showed remarkable antiproliferative activity with IC_{50} (0.0150 and 0.0170 μM), while compound **3b** with IC_{50} (0.0300 μM) showed only one third of their activity. Concerning compounds **4a-f**; Compounds **4a-d** revealed a higher potency than compounds **4e** and **4f.** Compounds **4a, 4b** and **4d** have nearly the same cytotoxic activity against HepG2 cell line with IC_{50} (0.0110, 0.0099, 0.0130, and 0.0102 μM). Also compounds **5a (**0.0130 μM) and **5b** (0.0110 μM) showed excellent activity as potent as compounds **4a-d.** Compound **6** disclosed very good antiproliferative activity (Table 1).

To summarize the cytotoxic activity of all the test compounds against both cell lines, compounds **2e, 3a, 3b, 3c, 4a-4d, 5a, 5b** and **6** showed broad spectrum excellent anticancer activity against both MCF-7 and HepG2 (Table 1).

Table 1
Results of *in vitro* cytotoxic activity of test compounds and colchicine against both MCF-7 and HepG2 cell lines.

Cpd. No.	R	R^1	R^2	MCF-7 (μM)	HepG2 (μM)
Colchicine				0.1504	0.1239
2a	3-OCH_3	Cl	–	0.1500	0.0540
2b	3-OCH_3	Br	–	0.1350	0.03400
2c	4-OCH_3	Br	–	0.1260	0.0300
2d	3,4,5-$(OCH_3)_3$	Cl	–	0.1200	0.0110
2e	3,4,5-$(OCH_3)_3$	Br	–	0.0770	0.0095
2f	3,4,5-$(OCH_3)_3$	F	–	0.1250	0.0619
2g	3,4,5-$(OCH_3)_3$	OCH_3	–	0.1030	0.0900
3a	3-OCH_3	Cl	–	0.0260	0.0150
3b	3-OCH_3	Br	–	0.0130	0.0300
3c	4-OCH_3	F	–	0.0140	0.0170
4a	3-OCH_3	Cl	C_6H_5	0.0097	0.0110
4b	3-OCH_3	Br	C_6H_5	0.0110	0.0099
4c	4-OCH_3	F	C_6H_5	0.0160	0.0130
4d	4-OCH_3	OCH_3	C_6H_5	0.0190	0.0102
4e	3-OCH_3	Cl	H	0.0490	0.0353
4f	3-OCH_3	Br	H	0.0490	0.0430
5a	OCH_3	Cl	–	0.0150	0.0130
5b	OCH_3	Br	–	0.0230	0.0110
6	–	–	–	0.0099	0.0372

2.3. Enzyme-linked immunosorbent assay for tubulin beta (TUBb)

This assay was employed to predict the possible mode of action of representative target compounds. The percentage inhibition of tubulin polymerization of representative of the most active target compounds against cancer cell lines was measured. Compounds **3b, 3c, 4a, 4b, 5a** and **6** (Fig. 3) with the highest cytotoxic activity against human breast cancer cell line (MCF-7) were tested for inhibiting tubulin beta (TUBb) polymerization using colchicine as a reference drug. All the selected tested compounds showed excellent TUBb polymerization inhibition activity. They were more potent than colchicine as TUBb polymerization inhibitors and this was in accordance with the *in vitro* cytotoxic activity of these compounds against MCF-7 cell line. Compounds **3b, 4a** and **6** (Fig. 3) were excellent TUBb polymerization inhibitors with percentage inhibition 92.62997%, 92.29742% and 92.16634% respectively, while the percentage of TUBb inhibition of colchicine was 86.2803% (Table 2).

All the selected test compounds with outstanding TUBb polymerization inhibition activity have almost the same common features; 3/4-methoxyphenyl (ring A), halo phenyl (ring C) and a bridge with a carbonyl group and/or a hydroxyl group (Fig. 3). Compounds **5a, 3c** and **4b** showed remarkable inhibition activity as well as great potencies against MCF-7.

In case of compounds that revealed excellent cytotoxic activity against human liver carcinoma (HepG2), TUBb polymerization inhibition assay was carried out for representative compounds **2a, 2c, 2d, 2e, 4a, 4b, 5a, 5b** and **6.** In spite of not being the most active compound amongst the representative target compounds, compound **4a** showed outstanding inhibition activity for TUBb polymerization with percentage inhibition 93.02209%. As they showed excellent cytotoxic activity against HepG2, compounds **2e, 2d, 2c** and **2a** (Fig. 4) showed very high percentage of TUBb polymerization inhibition (92.77821%, 92.36795%, 91.43021% and 90.58723%) much more potent than colchicine (82.52266%). Compounds **5a, 6** and **4b** were more potent as TUBb polymerization inhibitors than colchicine (Table 2).

2.4. Effect of representative target compounds on normal human breast cells and hepatic cells

One of the main problems of cancer chemotherapy is the unwanted damage to normal cells caused by the high toxicities of anticancer drugs. Representative target compounds that were the most active against MCF-7 and HepG2 and cause the highest percentage inhibition of TUBb polymerization were selected to be tested against normal breast cell line (Hs 371.T) and normal liver (AML12) cell line and their IC_{50} were determined using colchicine

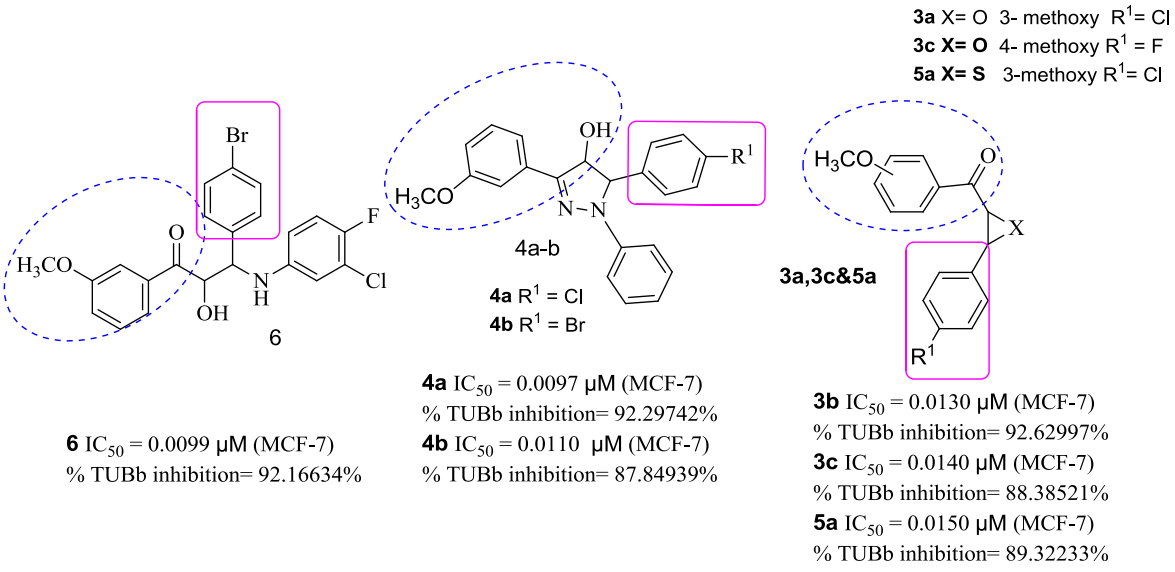
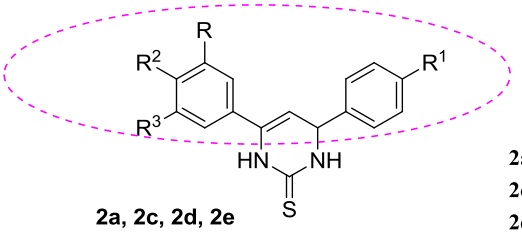


Fig. 3. Active compounds against MCF-7 cell line.

Table 2
Percentage inhibition of tubulin-b polymerization of selected target compounds.

Cpd. No.	MCF-7 (μM)	Tubulin % inhibition	Cpd. No.	HepG2 (μM)	Tubulin % inhibition
Colchicine	0.1504	86.2803	Colchicine	0.1239	82.52266
3b	0.0130	92.62997	**2a**	0.0540	90.58723
3c	0.0140	88.38521	**2c**	0.0300	91.43021
4a	0.0097	92.29742	**2d**	0.0110	92.36795
4b	0.0110	87.84939	**2e**	0.0095	92.77821
5a	0.0150	89.32233	**4a**	0.0110	93.02209
6	0.0099	92.16634	**4b**	0.0099	84.57606
			5a	0.0130	88.63453
			5b	0.0110	85.71133
			6	0.0372	88.4942



	R	R^1	R^2	R^3
2a	H	Cl	H	OCH_3
2c	H	Br	OCH_3	H
2d	OCH_3	Cl	OCH_3	OCH_3
2e	OCH_3	Br	OCH_3	OCH_3

2a IC_{50} = 0.0540 μM (HepG2)
% TUBb inhibition= 90.58723%
2c IC_{50} = 0.0300 μM (HepG2)
% TUBb inhibition= 91.43021%
2d IC_{50} = 0.0110 μM (HepG2)
% TUBb inhibition= 92.36795%
2e IC_{50} = 0.0095 μM (HepG2)
% TUBb inhibition= 92.77821%

Fig. 4. Active compounds against Hep G2 cell line.

as reference drug. Since all the target compounds were more potent against HepG2, six representative compounds were evaluated for their toxicity towards AML12 using colchicine as reference drug. Furthermore, three representative target compounds were evaluated for their cytotoxic effect on Hs 371.T (Breast N cells) via MTT assay. It was worthy to mention that, the IC_{50} doses of all the representative target compounds against normal breast and liver cells were very high in comparison to their IC_{50}s doses of the cancer cell lines, 2.5–9 folds more than the anticancer does (Table 3).

3. Conclusion

In this study, a group of novel compounds were synthesized according to structure based-drug design to be colchicine site

inhibitors (CSI). Compounds were tested for their cytotoxic activity against MCF-7 and HepG2 cell lines. Target compounds exhibited potent inhibitory activity at nanomolar level. Generally, target compounds showed more potent activity against HepG2 cell line than MCF-7 cell line. Target compounds **2e, 3a, 3b, 3c, 4a-4d, 5a, 5b** and **6** showed broad spectrum excellent anticancer activity against both MCF-7 and HepG2. Inhibition of TUBb polymerization test was used to confirm the mechanism of action of the representative broad spectrum antiproliferative active compounds. Compound **4a** showed the most TUBb inhibition activity. It is worthy to mention that, the replacement of either the enone or ethylenic bridge of the chalcone with rigid heterocyclic moieties resulted in superior antitumor potential ligands that may eventually allow the design and synthesis of chalcone derivatives with superior anticancer activity. The effect of the most active target compounds against normal breast cells and normal hepatic cells was tested and it was clear that the IC_{50} doses of the target compounds against cancer cell lines were safe doses for normal cells.

4. Experimental section

4.1. Chemistry

Melting points were determined on Stuart apparatus and the values given are uncorrected. Column Chromatography was used for purification by using solvent system benzene-acetone 90% (9:1). IR spectra were determined on Shimadzu IR 4000s spectrophotometer (KBr, cm^{-1}) at Faculty of Pharmacy, Misr University for Science and Technology, Egypt. ^{1}HNMR spectra were carried out using a Mercury, a Gemini 300-BB 300 MHz and Joel (eca), 300 or 400 MHz spectrophotometers using TMS as internal standard. Chemical shift values were recorded in ppm on δ scale, Microanalytical center, Cairo University, Egypt, Main laboratory of the War Chemical, Chemical War Department, Ministry of Defense and National Research Center, and Faculty of Pharmacy, Cairo University. ^{13}CNMR spectra were carried out using Mercury and Gemini 300-BB 75–100 MHz spectrophotometers using TMS as internal standard. Chemical shift values were recorded in ppm on δ scale, Micro analytical center, Cairo University, Egypt, and Faculty of Pharmacy, Cairo University. Mass spectra were recorded on Hewlett Packard 5988 spectrometer, Microanalytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Microanalytical Center, Faculty of Pharmacy, Al Azhar University, Egypt. Scientific microwave MSA2 was used irradiated under 300–400 W microwave irradiation, operating at 2450 Hz intermittently at 60 °C. Progress of the reactions was monitored using TLC sheets precoated with UV fluorescent silica gel Merck 60F 254. The solvent system was benzene, benzene: acetone and benzene: acetone: chloroform: TEA with different ratios and spots were visualized using UV lamp. The docking was performed using Auto Dock empirically derived program. Evaluation of the cytotoxic activity was performed at the Egyptian National Cancer Institute using MCF-7 and HPEG2 cell lines. Enzyme essay was carried out at VACSERA, Giza, Egypt using SEB870Hu 96 Tests Enzyme-linked Immunosorbent Assay Kit for Tubulin Beta (TUBb). Compounds **1a-i** and **3d** were prepared according to reported methods [14,26].

4.1.1. General procedure for preparation of compounds 2a-g

A mixture of compounds **1a, 1b, 1e, 1f, 1g, 1h** or **1i** (0.02 mol) and thiourea (1.52 g, 0.02 mol) in ethanolic sodium hydroxide (8%, 10 mL) was placed in a conical flask. The conical flask was covered with a funnel and then the flask was placed in a scientific microwave MSA2. The reaction mixture was irradiated under 300–400 W, operating at 2450 Hz intermittently at 60 °C for 3–4 h.

4.1.1.1. 4-(4-Chlorophenyl)-6-(3-methoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2a). Yield, 88%; mp 188–200 °C; IR (KBr) ν = 3394 (NH), 3183, 3073 (aromatic CH) cm^{-1}. ^{1}H NMR (DMSO-d_6) ppm δ: 3.82 (s, 3H, OCH_3), 5.14, 5.41 (2d, 1H, J = 2.56, 4.76 Hz), 6.89 (d, 1H, J = 7.92 Hz), 6.97–7.47 (m, 8H, Ar–H), 9.14 (s, 1H, NH, D_2O exchangeable), 9.28, 9.92 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) ppm δ: 175.7, 173.8, 159.6, 140.0, 135.1, 134.9, 129.9, 129.1, 128.8, 118.7, 115.5, 114.5, 111.4, 60.5, 55.6. GCMS m/z (% rel. abundance): 330.05 (M^+, 100%). Anal. Calcd for $C_{17}ClH_{15}N_2OS$ (330.83): C, 61.72; H, 4.57; N, 8.47. Found: 61.52; H, H 4.53; N, 8.51.

4.1.1.2. 4-(4-Bromophenyl)-6-(3-methoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2b). Yield, 85%; mp 212–214 °C; IR (KBr) ν = 3392 (NH), 3197 (aromatic CH) cm^{-1}. ^{1}H NMR (DMSO-d_6) ppm δ: 3.85 (s, 3H, OCH_3), 5.13, 5.42 (2d, 1H, J = 4.36, 4.44 Hz) 6.92 (d, 1H, J = 7.96 Hz) 7.00–7.99 (m, 8H, Ar–H), 9.13, 9.93 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) ppm δ: 173.7, 159.6, 143.2, 137.7, 135.0, 131.7, 130.3, 129.7, 122.1, 120.0, 115.4, 112.4, 56.5, 60.6, 54.1. GCMS m/z (% rel. abundance): 374.90 (M^+, 100%). Anal. Calcd for $C_{17}BrH_{15}N_2OS$ (375.27): C, 54.41; H, 4.03; N, 7.46. Found: 54.57; H, 4.01; N, 7.52.

4.1.1.3. 4-(4-Bromophenyl)-6-(4-methoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2c). Yield, 50%; mp 210–212 °C; IR (KBr) ν = 3399 (NH), 3189 (aromatic C–H) cm^{-1}. ^{1}H NMR (DMSO-d_6) ppm δ: 3.81 (s, 3H, OCH_3), 5.09, 5.27 (2d, 1H, J = 4.68, 4.92 Hz) 6.89 (d, 1H, J = 4.92 Hz), 7.01–7.67 (m, 8H, Ar–H), 9.05, 9.88 (2s, 2H, 2NH, D_2O exchangeable). GCMS m/z (% rel. abundance): 375.95 (M^+, 43.07%), 219.00 (100%). Anal. Calcd for $C_{17}BrH_{15}N_2OS$ (375.28): C, 54.41; H, 4.03; N, 7.46. Found: 54.60; H, 4.06; N, 7.41.

4.1.1.4. 4-(4-Chlorophenyl)-6-(3,4,5-trimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2d). Yield, 50%; mp 185–187 °C; IR (KBr) ν = 3398 (NH), 3196 (aromatic CH) cm^{-1}. ^{1}H NMR (DMSO-d_6) ppm δ: 3.78, 3.82 (2s, 9H, $3OCH_3$), 5.14, 5.45 (2d, 1H, J = 3.48, 4.52 Hz) 6.82 (d, 1H, J = 4.52 Hz), 6.94–8.39 (d, 6H, Ar–H), 9.00, 9.94 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) ppm δ: 175.5, 173.7, 153.1, 152.8, 142.8, 138.4, 134.8, 132.6, 129.1, 128.8, 123.0, 112.5, 103.8, 100.8, 60.5, 56.4. GCMS m/z (% rel. abundance): 332.00 (100%). Anal. Calcd for $C_{19}ClH_{19}N_2O_3S$ (390.88): C, 58.38; H, 4.90; N, 7.17. Found: 58.66; H, 4.61; N, 7.15.

4.1.1.5. 4-(4-Bromophenyl)-6-(3,4,5-trimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2e). Yield, 55%; mp 208–210 °C; IR (KBr) ν = 3389 (NH), 3176 (aromatic CH) cm^{-1}. ^{1}H NMR (DMSO-d_6) ppm δ: 3.73, 3.82 (2s, 9H, $3OCH_3$), 5.21, 5.44 (2d, 1H, J = 2.32, 4.36 Hz), 6.81 (d, 1H, J = 4.68 Hz), 7.23–7.62 (m, 6H, Ar–H), 9.11,

Table 3
Effect of colchicine and representative target compounds on normal breast cells and normal hepatic cells.

Cpd. No.	MCF-7 (μM)	Hs 371.T *(Breast N cells)* (μM)	Cpd. No.	HepG2 (μM)	AML 12 *(Liver N cells)* (μM)
Colchicine	0.1504	0.4434	Colchicine	0.1239	0.1381
4a	0.0097	0.0867	**2d**	0.011	0.0885
4b	0.011	0.4743	**2e**	0.0095	0.0594
6	0.0099	0.0902	**4a**	0.011	0.0741
			4b	0.0099	0.0269

9.93 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) ppm δ: 175.5, 153.2, 143.9, 138.4, 134.7, 132.1, 129.2, 129.0, 121.1, 103.7, 100.7, 60.4, 56.4, 54.6. GCMS *m/z* (% rel. abundance): 435.95 (M^+, 51.78%), 279.05 (100%). Anal. Calcd for $C_{19}ClH_{19}N_2O_3S$ (435.33): C, 52.42; H, 4.40; N, 6.43. Found: 52.65; H, 4.10; N, 6.63.

4.1.1.6. 4-(4-Flurophenyl)-6-(3,4,5-trimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2f). Yield, 60%; mp 179–181 °C; IR (KBr) ν = 3399 (NH), 3191 (aromatic C–H) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.77, 3.82, 3.91 (3s, 9H, $3OCH_3$), 5.14, 5.45 (2d, 1H, *J* = 2.48, 4.44 Hz), 6.81 (d, 1H, *J* = 3.76 Hz, pyrimidine), 7.08–7.44 (m, 6H, Ar–H), 8.98, 9.85 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) ppm δ: 175.4, 163.3, 160.9, 153.1, 140.8, 138.4, 134.7, 128.9, 115.8, 103.7, 101.0, 60.5, 56.4, 54.4. GCMS *m/z* (% rel. abundance): 374.05 ((M^+ 100%). Anal. Calcd for $C_{19}FH_{19}N_2O_3S$ (374.43): C, 60.95; H, 5.11; N, 7.48. Found: 60.77; H, 5.10; N, 7.73.

4.1.1.7. 4-(4-Methoxyphenyl)-6-(3,4,5-trimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2g). Yield, 95%; mp 216–218 °C; IR (KBr) ν = 3395 (NH), 3171 (aromatic C–H) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.82, 3.84, 3.90 (3s, 12 H, $4OCH_3$), 5.05, 5.41 (2d, 1H, *J* = 4.00, 4.68 Hz) 6.81 (d, 1H, *J* = 4.68 Hz), 6.94–7.23 (m, 6 H, Ar–H), 9.02, 9.83 (2s, 2H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) ppm δ: 175.1, 159.2, 153.0, 138.3, 136.7, 134.4, 129.2, 128.3, 114.4, 106.5, 103.7, 101.4, 60.4, 56.3, 55.6, 54.7. GCMS *m/z* (% rel. abundance): 386.05 (M^+, 100%). Anal. Calcd for $C_{20}H_{22}N_2O_4S$ (386.46): C, 62.16; H, 5.74; N 7.25. Found: 62.55; H, 5.69; N, 7.27.

4.1.2. General procedure for preparation of compounds 3a-c

To a well stirred solution of chalcone **1a, 1b, 1c or 1d** (0.04 mol) in a mixture of acetone (25 mL) and methanol (10 mL) at 0 °C, a solution of hydrogen peroxide (28%, 10 mL) in sodium hydroxide solution (4 N, 5 mL) was added over a period of time. After completion of addition, the reaction mixture was stirred for an additional one hour, and then poured onto ice-cold water (100 mL). The separated product was collected by filtration, washed with water and crystallized from ethanol to give compounds **3a-d**.

4.1.2.1. [3-(4-Chlorophenyl)oxiran-2-yl](3-methoxyphenyl)methanone (3a). Yield, 95%; reaction time 1 h; mp 90–92 °C; IR (KBr) ν = 3462- 3446 (C–H of oxirane ring), 1689 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.80 (s, 3H, OCH_3), 4.16 (d, 1H, *J* = 2.1 Hz), 4.82 (d, 1H, *J* = 2.1 Hz), 7.24–7.63 (m, 8H, Ar–H). ^{13}C NMR (DMSO-d_6) ppm δ: 192.6, 159.5, 136.5, 134.6, 133.5, 130.1, 128.5, 128.2, 120.8, 120.0, 112.6, 59.7, 57.9, 55.3. GCMS *m/z* (% rel. abundance): 287.90 ($M-1^+$, 12.57%), 135.00 (100%). Anal. Calcd for C_{16} Cl $H_{13}O_3$ (288.73): C, 66.56; H, 4.54. Found: C, 66.71; H, 4.59.

4.1.2.2. [3-(4-Bromophenyl)oxiran-2-yl](3-methoxyphenyl)methanone (3b). Yield, 95%; reaction time 1 h; mp 93–95 °C; IR (KBr) ν = 3446–3421 (C–H of oxirane ring), 1689 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.81 (s, 3H, OCH_3), 4.15 (d, 1H, *J* = 2.1 Hz), 4.82 (d, 1H, *J* = 1.8 Hz), 7.29–7.63 (m, 8H, Ar–H). GCMS *m/z* (% rel. abundance): 333.90 (M^+, 8.61%), 135.05 (100%). Anal. Calcd for C_{16} Br $H_{13}O_3$ (333.18): C, 57.68; H, 3.93. Found: 57.80; H, 3.91.

4.1.2.3. [3-(4-Flurophenyl)oxiran-2-yl](4-methoxyphenyl)methanone (3c). Yield, 71%; reaction time 4 h; mp 86–88 °C; mp 75–77; IR (KBr) ν = 3462–3446 (C–H of oxirane ring), 1680 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.85 (s, 3H, OCH_3), 4.13 (d, 1H, *J* = 2.4 Hz), 4.76 (d, 1H, *J* = 2.4 Hz), 7.06–7.27 (m, 4H, Ar–H), 7.47–7.52 (m, 2H, Ar–H), 8.01–8.04 (m, 2H, Ar–H). GCMS *m/z* (% rel. abundance): 272.00 (M^+, 5.65%), 135.05 (100%). Anal. Calcd for C_{16} F $H_{13}O_3$ (272.27): C, 70.58; H, 4.81. Found: 70.69; H, 4.89.

4.1.3. General procedure for preparation of compounds 4a-f

A solution of Oxirane **3a, 3b, 3c** or **3d** (0.02 mol) in absolute ethanol (30 mL) was refluxed with either phenyl hydrazine (0.02 mol, 1.97 mL) or hydrazine hydrate (99%, 0.02 mol, 0.64 mL) for 1–2 h. The solvent was evaporated under reduced pressure and the residue was purified either by crystallization from ethanol/ether mixture (2:1) for compounds **4a, 4b** and **4e** or by column chromatography using solvent system benzene: acetone (90%) for compounds **4c, 4d** and **4f**.

4.1.3.1. 5-(4-Chlorophenyl)-3-(3-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-4-ol (4a). Yield, 86%; mp 80–82 °C; IR (KBr) ν = 3317 (OH), 2951–2931 (aliphatic CH), 1597 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.86 (s, 3H, OCH_3), 5.07 (dd, 1H, *J* = 2.70, 3.00 Hz), 5.24 (d, 1H, *J* = 2.4 Hz), 6.52 (d, 1H, *J* = 7.5 Hz, OH, D_2O exchangeable), 6.80–7.50 (m, 13H, Ar–H). ^{13}C NMR (DMSO-d_6) ppm δ: 159.4, 147.9, 143.3, 137.9, 132.9, 132.2, 129.6, 129.0, 127.7, 119.2, 118.5, 114.3, 112.9, 111.0, 81.8, 71.7, 55.1. GCMS *m/z* (% rel. abundance): 379.30 ($M+1^+$, 0.77%), 378.30 (M^+, 1.61%), 81.00(100%). Anal. Calcd for C_{22} Cl $H_{19}N_2O_2$ (378.85): C, 69.75; H, 5.05; N, 7.39. Found: 69.92; H, 5.12; N, 7.53.

4.1.3.2. 5-(4-Bromophenyl)-3-(3-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-4-ol (4b). Yield, 83%; mp 82–84 °C; IR (KBr) ν = 3311 (OH), 3041 (aliphatic CH), 1593 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.86 (s, 3H, OCH_3), 5.09 (d, 1H, *J* = 7.20 Hz), 5.26 (s, 1H), 6.53 (d, 1H, *J* = 7.50 Hz, OH, D_2O exchangeable), 6.81–7.61 (m, 13H, Ar–H). GCMS *m/z* (% rel. abundance): 423.90 (M^+, 5.37%), 404. 00 (100%). Anal. Calcd for C_{22} Br $H_{19}N_2O_2$ (423.30): C, 62.42; H, 4.52; N, 6.62. Found: 62.58; H, 4.55; N, 6.71.

4.1.3.3. 5-(4-Flurophenyl)-3-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-4-ol (4c). Yield, 63%; mp 60–62 °C; IR (KBr) ν = 3446 (OH), 2933 (aliphatic CH) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.86, 3.92 (2s, 3H, OCH_3), 5.07 (s, 1H), 5.19 (s, 1H), 6.44 (d, 1H, OH, *J* = 7.2 Hz, D_2O exchangeable), 6.80–7.86 (m, 13H, Ar–H). GCMS *m/z* (% rel. abundance): 363.05 ($M+1^+$, 19.80%), 362.05 (M^+, 74.70%), 77.00 (100%). Anal. Calcd for C_{22} F $H_{19}N_2O_2$ (362.40): C, 72.91; H, 5.28; N, 7.73. Found: 73.12; H, 5.32; N, 7.91.

4.1.3.4. 5-(4-Methoxyphenyl)-3-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-4-ol (4d). Yield, 54%; mp 66–68 °C; IR (KBr) ν = 3394 (OH), 2931 (aliphatic CH), 1597 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.77 (s, 3H, OCH_3), 3.86, 3.91 (2s, 3H, OCH_3), 5.02 (s, 1H), 5.12 (s, 1H), 6.40 (d, 1H, OH, *J* = 6.3 Hz, D_2O exchangeable), 6.79–7.86 (m, 13H, Ar–H). GCMS *m/z* (% rel. abundance): 374.10 (M^+, 3.48%), 77.00 (100%). Anal. Calcd for $C_{18}H_{22}N_2O_3$ (374.43): C, 73.78; H, 5.92; N, 7.48. Found: 74.04; H, 5.99; N, 7.56.

4.1.3.5. 5-(4-Chlorophenyl)-3-(3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-4-ol (4e). Yield, 71%; mp 136–138 °C; IR (KBr) ν = 3444–3282 (NH and OH), 1598 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.81, 3.82 (s, 3H, OCH_3), 4.53 (d, 1H, *J* = 6.30 Hz), 4.92 (d, 1H, *J* = 6.3 Hz), 6.01 (d, 1H, *J* = 7.80 Hz, OH, D_2O exchangeable), 6.84–7.48 (m, 8H, Ar–H), 7.77 (d, 1H, *J* = 3.0 Hz, NH, D_2O exchangeable). GCMS *m/z* (% rel. abundance): 304.20 ($M+2^+$, 0.57%), 302.20 (M^+, 1.06%), 75.05 (100%). Anal. Calcd for C_{16} Cl $H_{15}N_2O_2$ (302.76): C, 63.47; H, 4.99; N, 9.25. Found: 63.65; H, 5.07; N, 9.33.

4.1.3.6. 5-(4-Bromophenyl)-3-(3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-4-ol (4f). Yield, 55%; mp 130–132 °C; IR (KBr) ν = 3435–2926 (NH and OH), 1577 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.84 (s, 3H, OCH_3), 4.13 (d, 1H, *J* = 8.1 Hz), 4.81 (s, 1H), 7.26 (d, 1H, *J* = 8.1 Hz, OH, D_2O exchangeable), 7.12–7.84 (m, 8H,

Ar–H), 8.69 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) ppm δ: 160.7, 158.4, 142.3, 136.9, 131.9, 127.7, 129.8, 120.8, 122.3, 116.3, 113.9, 83.9, 56.9, 55.9. GCMS *m/z* (% rel. abundance): 347.10($M+^{\cdot+}$, 3.91%), 135.10(100%). Anal. Calcd for C_{16} Br $H_{15}N_2O_2$ (347.21): C, 55.38; H, 4.35; N, 8.07. Found: 55.46; H, 4.39; N, 8.14.

4.1.4. General procedure for preparation of compounds 5a-b

Compounds **5a** and **5b** were prepared adopting the same procedure used for compounds **4a-f** using **3a** or **3b** as starting materials, and thiourea (1.52 g, 0.02 mol) as a cycling agent instead of phenyl hydrazine or hydrazine hydrate. Compounds **5a-b** were crystallization from a mixture of ethanol/ether (2:1).

4.1.4.1. (3-(4-Chlorophenyl)thiiran-2-yl)(3-methoxyphenyl)methanone (5a). Yield, 58%; mp 101–103 °C; IR (KBr) $\nu = 3059$ (aromatic CH), 1685 (C=O), 1263 cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.80 (s, 3H, OCH_3), 4.16 (d, 1H, $J = 2.8$ Hz), 4.82 (d, 1H, $J = 2.8$ Hz), 7.26–7.64 (m, 8H, Ar–H). ^{13}C NMR (DMSO-d_6) ppm δ: 192.5, 159.4, 136.5, 134.6, 133.4, 130.5, 129.8, 128.4, 120.7, 119.9, 112.6, 59.7, 57.8, 55.3. Anal. Calcd for $C_{16}Cl$ $H_{13}O_2S$ (304.79): C, 63.05; H, 4.30; Found: 63.04; H, 4.34.

4.1.4.2. (3-(4-Bromophenyl)thiiran-2-yl)(3-methoxyphenyl)methanone (5b). Yield, 50%; mp 106–108 °C; IR (KBr) $\nu = 3060$ (aromatic CH), 1664 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.81 (s, 3H, OCH_3), 4.15 (d, 1H, $J = 2.4$ Hz), 4.81 (d, 1H, $J = 2.4$ Hz), 7.26–7.63 (m, 8H, Ar–H). Anal. Calcd for $C_{16}BrH_{13}O_2S$ (349.24): C, 55.03; H, 3.75. Found: 55.26; H, 3.89.

4.1.5. 3-(4-Bromophenyl)-3-[(3-chloro-4-fluorophenyl)amino]-2-hydroxy-1-(3-methoxyphenyl) propan-1-one (6)

A solution of oxirane **3b** (6.66 g, 0.02mol) in absolute ethanol (20 mL) was heated with 3-chloro-4-fluro-aniline (5.82 g, 0.04mol) for 2 h. The reaction mixture was filtered while hot, concentrated under vacuum and then cooled. The separated product was filtered and crystallized from ethanol to give compound **6**.

Yield, 77%; mp 136–138 °C; IR (KBr) $\nu = 3408–3358$ (OH, NH), 1677 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) ppm δ: 3.80 (s, 3H, OCH_3), 4.78 (d, 1H, $J = 8.40$ Hz), 5.04 (t, 1H), 5.05 (d, 1H, $J = 8.40$ Hz), 5.84 (d, 1H, $J = 8.40$ Hz, NH, D_2O exchangeable), 6.36 (d, 1H, $J = 12.4$ Hz, OH, D_2O exchangeable), 6.45–6.62 (m, 1H, Ar–H), 6.62 (dd, 1H, $J = 8.40$ Hz, $J = 8.40$ Hz, Ar–H), 6.96–7.02 (m, 1H, Ar–H), 7.20–7.61 (m, 8H, Ar–H). ^{13}C NMR (DMSO-d_6) ppm δ: 197.2, 160.3, 147.3, 144.4, 142.7, 134.6, 131.6, 129.3, 127.8, 122.5, 121.9, 121.5, 118.7, 117.0, 115.5, 114.5, 90.5, 63.9, 55.7. GCMS *m/z* (% rel. abundance): 481.30 ($M + 3^{\cdot+}$, 15.99%), 480.30 ($M + 2^{\cdot+}$, 19.19%), 479.30 ($M + 1^{\cdot+}$, 15.70%), 478.30 ($M+^{\cdot+}$, 27.33%), 337.30 (100%). Anal. Calcd for $C_{22}BrClFH_{18}NO_3$ (478.74): C, 55.19; H, 3.79; N, 2.93. Found: 55.34; H, 3.81; N, 2.97.

4.2. Cytotoxic activity

The newly synthesized compounds were evaluated on the *in-vitro* growth inhibition of two solid human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7) and liver hepatocellular carcinoma (HepG2) using colchicine as reference drug using SRB assay. Moreover, the toxic effect of representative target compounds was evaluated on normal breast (Hs. 371.T) and liver (AML 12) cell lines using MTT assay.

4.2.1. Cell cultures

The antitumor activity was determined for the newly synthesized compounds in Egyptian National Cancer Institute (NCI) for *in-vitro* detection of IC_{50} of their antitumor activity. The breast tumor cell line (MCF-7) and hepatic tumor cell line HepG2 were obtained frozen in liquid nitrogen (−180 °C) from the American Type Culture Collection (ATCC) and was maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. All chemicals used in this study are of high analytical grade. They were obtained from (either Sigma-Aldrich or Bio-Rad).

The cytotoxicity of the test compounds was determined using SRB assay applying the method of Skehan et al. [27]. These testing procedures were carried out at pharmacology lab at Cancer Biology Unit in Egyptian National Cancer Institute. The Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow the attachment of cells to the wall of the plate. Different concentrations of each compound (0, 1, 2.5, 5 and 10 μg/mL) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line, The IC_{50} value was calculated using sigmoidal dose response curve-fitting models (Graph Pad, Prizm software incorporated), each concentration was repeated 3 times (Table 1).

4.3. Tubulin enzyme assay

MCF-7 and HepG2 cell lines were obtained from American Type Culture Collection, they were cultured using DMEM (Invitrogen/Life Technologies) supplemented with 10% FBS (Hyclone), 10 μg/mL of insulin (Sigma), and 1% penicillin-streptomycin. Plate cells (cells density 1.2–1.8 × 10,000 cells/well) in a volume of 100 μl complete growth medium and 100 μl of the tested compound per well in a 96-well plate for 18–24 h before the enzyme assay for Tubulin. The microtiter plate provided in this kit has been pre-coated with an antibody specific to TUBb. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to TUBb. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain TUBb, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 10 nm. The concentration of TUBb in the samples is then determined by comparing the O.D. of the samples to the standard curve [28].

References

[1] K. Nepali, S. Sharma, M. Sharma, P.M.S. Bedi, K.L. Dhar, Rational approaches, design strategies, structure activity relationship and mechanistic insights for anticancer hybrids, Eur. Med. Chem. 77 (2014) 422–487.

[2] A. Petrelli, S. Giordano, From single- to multi-target drugs in cancer therapy: when a specificity becomes an advantage, Curr. Med. Chem. 15 (2008) 422–432.

[3] G. De Martino, M.C. Edler, G. La Regina, A. Coluccia, M.C. Barbera, D. Barrow, R.I. Nicholson, G. Chiosis, A. Brancale, E. Hamel, M. Artico, R. Silvestri, New Arylthioindoles: potent inhibitors of tubulin polymerization. 2. Structure-activity relationships and molecular modeling studies, J. Med. Chem. 49 (2006) 947–954.

[4] B.L. Flynn, G.S. Gill, D.W. Grobelny, J.H. Chaplin, D. Paul, A.F. Leske, T.C. Lavranos, D.K. Chalmers, S.A. Charman, E. Kostewicz, D.M. Shackleford, J. Morizzi, E. Hamel, M.K. Jung, G. Kremmidiotis, Discovery of 7-hydroxy-6-methoxy-2-methyl-3-(3,4,5-trimethoxybenzoyl)benzo[*b*]furan (BNC105), a tubulin polymerization inhibitor with potent antiproliferative and tumor vascular disrupting properties, J. Med. Chem. 54 (2011) 6014–6027.

[5] X. Zhang, Y. Kong, J. Zhang, M. Su, Y. Zhou, Y. Zang, J. Li, Y. Chen, Y. Fang, X. Zhang, W. Lu, Design, synthesis and biological evaluation of colchicine

derivatives as novel tubulin and histone deacetylase dual inhibitors, Eur. Med. Chem. 95 (2015) 127–135.
[6] M. Botta, S. Forli, M. Magnani, F. Manetti, Molecular modeling approaches to study the binding mode on tubulin of microtubule destabilizing and stabilizing agents, Top. Curr. Chem. 286 (2009) 279–328.
[7] Y.-N. Shen, L. Lin, H.-Y. Qiu, W.-Y. Zou, Y. Qian, H.-L. Zhu, The design, synthesis, *in vitro* biological evaluation and molecular modeling of novel benzenesulfonate derivatives bearing chalcone moieties as potent anti-microtubulin polymerization agents, RSC Adv. 5 (2015) 23767–23777.
[8] P.M. Ranjit, S. Abdul-Rahman, K.P. Kumar, Y.R. Prasad, T. Santhipriya, G.C.V.S. Manikanta, N.L. Sudeepthi, Synthesis, screening and *in vitro* anticancer activity of piperazine nucleus containing novel chalcones on different cell lines, Int. J. Pharma. Tech. 5 (2013) 284–293.
[9] S. Syam, S.I. Abdelwahab, M.A. Al-Mamary, S. Mohan, Synthesis of chalcones with anticancer activities, Molecules 17 (2012) 6179–6195.
[10] C. Dyrager, M. Wickström, M. Fridén-Saxin, A. Friberg, K. Dahlén, E.A.A. Wallén, J. Gullbo, M. Grøtli, K. Luthman, Inhibitors and promoters of tubulin polymerization: synthesis and biological evaluation of chalcones and related dienones as potential anticancer agents, Bioorg. Med. Chem. 19 (2011) 2659–2665.
[11] Y. Shan, J. Zhang, J. Liu, M. Wang, Y. Dong, Development of combretastatin A-4 derivatives as anticancer agents, Curr. Med. Chem. 18 (2011) 523–538.
[12] H. Han, Y. Zhao, T. Cuthbertson, R. F. Hartman, S. D. Rose, Cell cycle arrest and apoptosis induction by an anticancer chalcone epoxide, Arch. Pharm. Chem. Life Sci., 8 (2010) 429–439, [ref importance of pyrazole moiety] D. Pal, S. Saha, S. Singh, Importance of Pyrazole Moiety in The Field of Cancer, Int J Pharm Pharm Sci, 4 (2012) 98–104.
[13] Y. Qian, G.-Y. Ma, Y. Yang, K. Cheng, Q.-Z. Zheng, W.-J. Mao, L. Shi, J. Zhao, H.-L. Zhu, Synthesis, molecular modeling and biological evaluation of dithiocarbamates as novel antitubulin agents, Bioorg. Med. Chem. 18 (2010) 4310–4316.
[14] S. Ducki, Antimitotic chalcones and related compounds as inhibitors of tubulin assembly, Anti-Cancer Agents Med. Chem. 9 (2009) 336–347.
[15] S. Ducki, The development of chalcones as promising anticancer agents, IDrugs 10 (2007) 42–46.
[16] C. Rappl, P. Barbier, V. Bourgarel-Rey, C. Gregoire, R. Gilli, M. Carre, S. Combes, P. J-Finet, V. Peyrot, Interaction of 4-arylcoumarin analogues of combretastatins with microtubule network of HBL100 cells and binding to tubulin, Biochemistry 45 (2006) 9210–9218.
[17] D.Y. Kim, K.-H. Kim, N.D. Kim, K.Y. Lee, C.K. Han, J.H. Yoon, S.K. Moon, S.S. Lee, B.L. Seong, Design and biological evaluation of novel tubulin inhibitors as antimitotic agents using a pharmacophore binding model with tubulin, J. Med. Chem. 49 (2006) 5664–5670.
[18] M.L. Edwards, D.M. Stemerick, P.S. Sunkara, Chalcones: a new class of antimitotic agents, J. Med. Chem. 33 (1990) 1948–1954.
[19] G. Herman, S. Busson, M.J. Gorbunoff, P. Mauduit, S.N. TimasheffIMA, B. Rossignol, Colchicine analogues that bind reversibly to tubulin define microtubular requirements for newly synthesized protein secretion in rat lacrimal gland, Cell Biol. 86 (1989) 4515–4519.
[20] S. Ducki, G. Mackenzie, B. Greedy, S. Armitage, J.F. Dit Chabert, E. Bennett, J. Nettles, J.P. Snyder, N.J. Lawrence, Combretastin-like chalcones as inhibitors of microtubule polymerization. Part 2: structure-based discovery of alpha-aryl chalcones, Bioorg. Med. Chem. 17 (2009) 7711–7722.
[21] R.S. Dongre, A.R. Bhat, J.S. Meshram, Anticancer activity of assorted annulated pyrimidine: a comprehensive review, Am. J. Pharm. Tech. Res. 4 (2014) 138–155.
[22] V.D. Joshi, M.D. Kshirsagar, S. Singhal, Synthesis and pharmacological study of some novel pyrimidines, Der Pharmacia Sinica 3 (2012) 343–348.
[23] D. Havrylyuk, O. Roman, R. Lesyk, Synthetic approaches, structure activity relationship and biological applications for pharmacologically attractive pyrazole/pyrazoline–thiazolidine-based hybrids, Eur. Med. Chem. 113 (2016) 145–166.
[24] R. Kant, D. Kumar, D. Agarwal, R.D. Gupta, R. Tilak, S.K. Awasthi, A. Agarwal, Synthesis of newer 1,2,3-triazole linked chalcone and flavone hybrid compounds and evaluation of their antimicrobial and cytotoxic activities, Eur. Med. Chem. 113 (2016) 34–49.
[25] B. Bhattacharyya, D. Panda, S. Gupta, M. Banerjee, Anti-mitotic activity of colchicine and the structural basis for its interaction with tubulin, Med. Res. Rev. 28 (2008) 155–183.
[26] L. Guo, C. Yang, L. Zheng, W. Xia, Visible light-mediated oxidative quenching reaction to electron-rich epoxides: highly regioselective synthesis of α-bromo(di)ketones and mechanism study, Org. Biomol. Chem. 11 (2013) 5787–5792.
[27] P. Skehan, A. Monk, D.A. Scudiero, R.H. Shoemaker, K.D. Paull, DTP, DCTD Tumor repository a catalog of *in vitro* cell lines, transplantable animal and human tumors and micro assays, J. Natl. Cancer Inst. 83 (1991) 757–766.
[28] K. Liliom, A. Lehotzky, A. Molnar, J. Ovadi, Characterization of tubulin-alkaloid interactions by enzyme-linked immunosorbent assay, Anal. Biochem. 228 (1995) 18–26.