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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Biological evaluation of synthetic analogues of curcumin: chloro-substituted-2′-hydroxychalcones as potential inhibitors of tubulin polymerization and cell proliferation

Hassan Aryapour · Gholam Hossein Riazi · Alireza Foroumadi · Shahin Ahmadian · Abbas Shafiee · Oveis Karima · Majid Mahdavi · Saeed Emami · Maedeh Sorkhi · Sirus Khodadady

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Abstract A series of chloro-substituted-2′-hydroxychalcones were prepared and evaluated for their cytotoxic effects against K562 and SK-N-MC human cancer cell lines and as the inhibitors of tubulin polymerization. The 3,5′-dichloro- analogue (compound **3**) inhibited the assembly of protofilaments with 89% inhibition. Compound **3** was found to be bound to tubulin with a dissociation constant of 3.7 μM and altered far-UV circular dichroism spectrum of tubulin and altered far-UV circular dichroism spectrum of tubulin.

Keywords Microtubule · Chalcone · Curcumin · K562 · SK-N-MC

Introduction

Microtubules are highly dynamic assemblies of $\alpha\beta$-tubulin. When cells enter mitosis, microtubule dynamics increase up to 100-fold as compared with those taking part in interphase (Jiang *et al*., 1998). High-speed dynamic is very sensitive to interferences and is, therefore, a moving target of many chemotherapeutic agents in tumor cells, such as curcumin, vinca alkaloids, chalcones, and paclitaxel (Rowinsky and Donehower, 1991; Tishler *et al*., 1995). The primary action of these compounds is to bind the sites on tubulin subunits or microtubules and then the alteration of microtubule architecture, dynamics and subsequently, mitotic blockage (Jordan and Wilson, 1998). In addition to binding with tubulin, several microtubule-targeting compounds have been found to alter dynamic stability of microtubules by binding to the microtubule associated proteins (e.g., estramustine), (Yoshida *et al*., 2000) and motor proteins (e.g., monastrol) (Mayer *et al*., 1999). Curcumin, a naturally occurring polyphenolic compound, is extracted from the rhizomes of *Curcuma longa*. Curcumin exists in equilibrium with its enol tautomer. The bis-keto form of curcumin predominates in acidic and neutral aqueous solutions and in the cell membrane (Wang *et al*., 1997). It has been used as an important dietary component for a long time (Aggarwal *et al*., 2007). It exhibits various biological functions including antiproliferative activity against various cancer cells, antiangiogenic, and antioxidant activity (Ruby *et al*., 1995; Gururaj *et al*., 2002), wound healing ability (Panchatcharam *et al*., 2006) and antimicrobial property (Rai *et al*., 2008). Recently, it has been found that curcumin binds to tubulin and perturbs microtubule polymerization. Curcumin inhibits the proliferation of HeLa and MCF-7 cells in a concentration-dependent manner with IC_{50} of 13.8 ± 0.7 and 12 ± 0.6 μM, respectively. At higher inhibitory concentrations (>10 μM), curcumin induces the significant depolymerization of interphase microtubules and mitotic spindle microtubules of HeLa and MCF-7 cells (Gupta

H. Aryapour · G. H. Riazi (✉) · S. Ahmadian · O. Karima · M. Mahdavi · S. Khodadady
Institute of Biochemistry and Biophysics, University of Tehran, Enghelab Ave, P.O. Box: 13145-1384, Tehran, Iran
e-mail: ghriazi@ibb.ut.ac.ir

A. Foroumadi · A. Shafiee · M. Sorkhi
Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

S. Emami
Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

et al., 2006). During the present study, we have designed and synthesized several new chalcone analogues of curcumin family with anti-microtubule properties and evaluate their cytotoxic activities against K562 and SK-N-MC human cancer cell lines.

Materials and methods

General

Curcumin was synthesized on the basis of previous procedures (Hoehle *et al.*, 2006). Briefly, acetylacetone was allowed to react with the substituted benzaldehyde and boric acid anhydride as a catalyst. According to HPLC procedure, the final products obtained after crystallization from methanol were >99% pure. Melting points were obtained using a Kofler hot stage apparatus and are uncorrected. The IR spectra were recorded on a Nicollet FT-IR Magna 550 spectrometer. The ^{1}H NMR spectra were recorded on a Bruker 400 spectrometer and chemical shifts are in ppm relative to internal tetramethylsilane (TMS). Guanosine-5′-triphosphate (GTP) and piperazine-1, 4-bis (2-ethanesulfonic acid) (PIPES) were purchased from Sigma-Aldrich. All other chemicals were of analytical grade. All of the test compounds, used in this study, were dissolved in DMSO (dimethyl sulfoxide) as 1 mM stock. For in vitro and cell culture tests, DMSO was used at the final concentrations of <4 and <0.1% (volume/volume), respectively. All solutions were stored in the refrigerator and protected from light. RPMI-1640 medium and MTT kit were purchased from Invitrogen and Roche Corporation, respectively. The K562 and SK-N-MC cancer cell lines were obtained from Pasture Research Center.

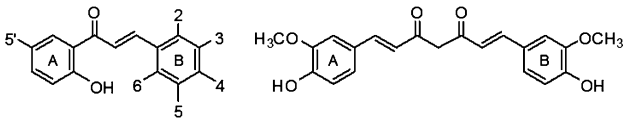
General procedure for synthesis of 2′-hydroxychalcones

All of the compounds **1**–**5**, presented in Table 1, were prepared according to the same general synthetic procedure with some modifications (Robinson *et al.*, 2003). A mixture of the aldehyde (10 mmol, 1 equiv.) and the appropriately substituted aromatic ketone (10 mmol, 1 equiv.) was dissolved in 30 ml methanol. One milliliter of 50% NaOH aqueous solution was added to the mixture and then allowed to stir overnight at room temperature. The precipitated solids were collected by suction filtration procedure. Recrystallization from ethanol provided the pure product. During the event when solid was not formed, the solution was neutralized with diluted HCl solution (1 N) and extraction was done in chloroform (2 × 50 ml). The combined organic layers were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residues were purified by column chromatography.

(E)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one (1)

Yield 85%; m.p. 89–91°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.8 (s, 1H, OH), 7.94 (d, 1H, $J = 14$ Hz, H alkene), 7.9–7.94 (m, 1H, H aromatic), 7.68 (d, 1H, $J = 14$ Hz, H alkene), 7.66–7.7 (m, 2H, H aromatic), 7.52 (t, 1H, $J = 8$ Hz, H aromatic), 7.42–7.48 (m, 3H, H aromatic), 7.04 (d, 1H, $J = 8.8$ Hz, H aromatic), 6.96 (t, 1H, $J = 7.2$ Hz, H aromatic). IR (KBr, cm^{-1}) ν_{max}: 3430 (OH), 1641 (C=O).

Table 1 Structure, inhibition of tubulin polymerization, and cytotoxicity activity of synthetic chalcones **1**–**5** in comparison with the natural compound, curcumin



Compound	5′	2	3	4	6	(%)		(IC50, μM)	
						Yield	Tublin inhibition	K562	SK-N-MC
1	H	H	H	H	H	85	49.3 ± 2.4	60.7 ± 2.6	61.2 ± 2.5
2	Cl	H	H	Cl	Cl	75	>50	65.6 ± 3.3	69.8 ± 3.8
3	Cl	H	Cl	H	H	75	19.6 ± 0.9	41.1 ± 1.2	47.8 ± 3.2
4	Cl	H	H	Cl	H	79	20.3 ± 0.5	38.7 ± 0.9	45.8 ± 1.6
5	Cl	Cl	H	H	H	77	21.4 ± 1.0	41.8 ± 1.3	51.3 ± 2.7
6	–	–	–	–	–	83	26.6 ± 1.0	49.2 ± 1.3[a]	57.4 ± 2.9

[a] According to (Roy *et al.*, 2002), compound **6** gave an IC_{50} of 54.3 μm on K562 cells. Values are the mean ± SD

(E)-1-(5-chloro-2-hydroxyphenyl)-3-(2,4-dichlorophenyl)prop-2-en-1-one (2)

Yield 75%; m.p. 144–148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.6 (s, 1H, OH), 8.26 (d, 1H, $J = 16$ Hz, H alkene), 7.84 (d, 1H, $J = 2.4$ Hz, H aromatic), 7.74 (d, 1H, $J = 2.4$ Hz, H aromatic), 7.54 (d, 1H, $J = 16$ Hz, H alkene), 7.51 (d, 1H, $J = 2.5$ Hz, H aromatic), 7.47 (dd, 1H, $J = 8.8$ and 2.8 Hz), 7.35 (dd, 1H, $J = 8.4$ and 1.6 Hz), 7.02 (d, 1H, $J = 9.2$ Hz, H aromatic). IR (KBr, cm^{-1}) ν_{max}: 3440 (OH), 1638 (C=O).

(E)-1-(5-chloro-2-hydroxyphenyl)-3-(3-chlorophenyl)prop-2-en-1-one (3)

Yield 75%; m.p. 146–147°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.6 (s, 1H, OH), 7.85 (d, 1H, $J = 2.4$ Hz, H aromatic), 7.84 (d, 1H, $J = 15.6$ Hz, H alkene), 7.67 (d, 1H, $J = 9.2$ Hz, H aromatic), 7.55 (d, 1H, $J = 15.2$ Hz, H alkene), 7.36–7.48 (m, 4H, H aromatic), 6.98 (d, 1H, $J = 9.2$ Hz, H aromatic). IR (KBr, cm^{-1}) ν_{max}: 3425 (OH), 1638 (C=O).

(E)-1-(5-chloro-2-hydroxyphenyl)-3-(4-chlorophenyl)prop-2-en-1-one (4)

Yield 79%; m.p. 131–133°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.7 (s, 1H, OH), 7.9 (d, 1H, $J = 15.2$ Hz, H alkene), 7.82–7.86 (m, 1H, H aromatic), 7.63 (d, 1H, $J = 8$ Hz, H aromatic), 7.55 (d, 1H, $J = 15.2$ Hz, H alkene), 7.4–7.5 (m, 4H, H aromatic), 7.01 (d, 1H, $J = 8.8$ Hz, H aromatic). IR (KBr, cm^{-1}) ν_{max}: 3435 (OH), 1646 (C=O).

(E)-1-(5-chloro-2-hydroxyphenyl)-3-(2-chlorophenyl)prop-2-en-1-one (5)

Yield 77%; m.p. 186–192°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.7 (s, 1H, OH), 8.35 (d, 1H, $J = 15.2$ Hz, H alkene), 7.89 (d, 1H, $J = 2.4$ Hz, H aromatic), 7.8 (d, 1H, $J = 7.2$ Hz, H aromatic), 7.56 (d, 1H, $J = 15.2$ Hz, H alkene), 7.42–7.52 (m, 2H, H aromatic), 7.32–7.43 (m, 2H, H aromatic), 7.01 (d, 1H, $J = 8.8$ Hz, H aromatic). IR (KBr, cm^{-1}) ν_{max}: 3470 (OH), 1643 (C=O).

MTP preparation and purification

Microtubule protein (MTP) was prepared and purified from fresh sheep brain through two cycles of polymerization–depolymerization in the PEM buffer (100 mM PIPES, pH 6.9, 1 mM $MgSO_4$, and 1 mM EGTA) (Sengupta *et al.*, 2005). Then, MAP-free tubulin was purified from the microtubule protein by DEAE chromatography, (Murphy *et al.*, 1977) aliquot and stored in liquid nitrogen when not in use. The protein content was estimated by Bradford method using BSA as a standard (Bradford, 1976). The composition of MTP and tubulin purity, before and after DEAE chromatography, was determined using 10% (w/v) SDS-PAGE (data not shown).

Microtubule assembly assay

The microtubule assembly was monitored spectroscopically by turbidity measurement at 350 nm using a spectrophotometer (Varian Cary 100 Bio UV-Visible Spectrometer, USA), equipped with a thermostatically regulated liquid circulator. The assay was carried out based on previous procedures with some modification (Ma *et al.*, 2008). Briefly, tubulin was incubated with DMSO and compounds at different concentrations for 15 min at 0°C in ice. After adding the final 1 mM concentration of GTP, the assembly was initiated by warming the solution from 0 to 37°C and polymerization process was monitored by observing the variations in absorbance at 350 nm. Turbidity (absorbance) readings were recorded after every 10 s throughout the incubation time and the changes in absorbance were used to calculate the extent of polymerization [% inhibition = $(1–A_{350}$ sample$/A_{350}$ control$) \times 100$].

Electron microscopic analysis

The electron microscopic pictures were taken as described by (Rai *et al.*, 2008). Tubulin (1 mg/ml) was polymerized in the absence and presence of different concentrations of compound **3** in the PEM buffer at 37°C. After 15 min of polymerization, the microtubule polymers (20 µl) were transferred to the formvar-carbon-coated copper grids (400 meshes) for 45 s and blotted dry. The grids were subsequently stained negatively with a 1% uranyl acetate solution for 35 s, air-dried and observed under transmission electron microscope (Hitachi HU-12A) at 15000× magnification.

Fluorescence measurements

All of the fluorescence measurements were performed in a Varian's Cary Eclipse fluorescence spectrophotometer. The increased fluorescence of compound **3**, at 425 nm, upon binding to tubulin was used to determine the ligand affinity with tubulin (Rai *et al.*, 2008; Gupta *et al.*, 2006). Tubulin (2 µm) was incubated with varying concentrations of ligand (0–10 µm) in 25 mM PIPES at 25°C for 30 min in the dark. The excitation wavelength was 371 nm. The binding constants and stoichiometries were determined from Scatchard equation by plotting L_{bound}/L_{free} against L_{bound}, where L_{bound} is the bound ligand concentration and

L_{free} is the free-ligand concentration (Scatchard, 1949). The L_{bound} was calculated from the following equation:

$$L_{bound} = L_{total}/Q - 1(F/F_0 - 1)$$

where, Q is the enhancement factor of compound **3** fluorescence for bound ligand. Q was measured by plotting 1/fluorescence intensity of ligand against 1/[tubulin]. For this, a fixed amount of compound **3** (1 μM) was incubated with the increasing amounts of tubulin in a concentration range of 1–12 μM. Where, F is the fluorescence intensity of the tubulin-ligand complex at a particular ligand concentration and F_0 is the fluorescence intensity of ligand, alone, in the absence of tubulin. The amount of free-ligand is obtained from the difference of total ligand and calculated bound ligand. The corrections of inner filter effect were performed using the following equation: $F_{corrected} = F_{observed} \times \text{antilog}\,[(OD_{ex} - OD_{em})/2]$.

Analysis of secondary structural changes

Tubulin (4 μM) was incubated in the absence or presence of different concentrations of compound **3** (25 and 50 μM) in 15 mM phosphate buffer (pH: 6.5) for 30 min at 25°C. The far-UV (215–250 nm) circular dichroism (CD) spectra were monitored at 25°C using Aviv spectropolarimeter (model 215), equipped with a Peltier temperature-control system using a 1-mm path length quartz cuvette. Deconvolution and data analysis of the CD spectra were performed using CDNN from Jasco and GraphPad Prism, respectively.

Cell growth inhibition assay

MTT colorimetric assay was employed according to the methods of (Scudiero *et al.*, 1988). The K562 and SK-N-MC human cancer cell lines were maintained in RPMI-1640 medium, supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 UI/ml penicillin, and 50 UI/ml streptomycin. Cells were grown at 37°C and 5% CO_2 in humidified atmosphere. Ten thousand cells/well were seeded in 200 μl of the growth medium in 96-well microtiter plates (SPL Life Science, Korea) and incubated for 24 h before addition of test drugs. The cells were treated with several concentrations of test compounds in a CO_2 incubator for 72 h. At the end of this period, 10 μl of MTT (final concentration 0.5 mg/ml) was added to each well and the plates were incubated for 4 h at 37°C. Finally, 100 μl of the solubilization solution was added into each well and overnighted at 37°C. Absorbance (A_{570}) was determined, at 570 nm, for each well using microplate reader (Model Expert 96, Asys Hitech, Ec. Austria). The IC_{50} (concentration reducing by 50% the absorbance at 570 nm) was calculated by linear regression, performed on the linear zone of the dose–response curve. Control wells contained all of the compounds presented in the treated wells except compounds. Each experiment was performed in three replicates.

Results

Effects of synthetic chalcones on the in vitro polymerization of microtubules

As microtubules, in cells, are associated with many microtubule-interacting proteins, we analyzed MTP assembly from sheep brain in the absence and presence of different concentrations of synthetic chalcones (10–50 μM). The assembly was monitored by spectrophotometeric method (Fig. 1). The IC_{50} values of polymerization inhibition were determined from the semi-logarithmic dose–response plots using nonlinear regression program SigmaPlot. Compounds **3**, **4**, and **5** exhibited more potent activity and inhibited the assembly of purified sheep brain MTP in a concentration-dependent manner with the IC_{50} values of ≤21.4 μM. Also,

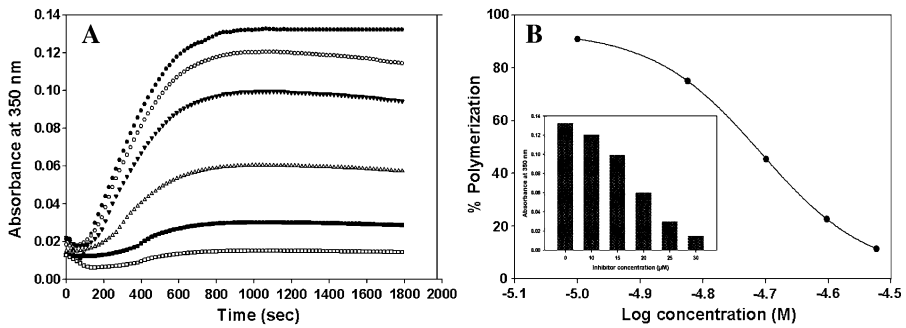


Fig. 1 Inhibition of tubulin polymerization by compound **3**. **a** Kinetics of the inhibition of microtubule assembly by increasing concentrations of compound **3**. Control (*filled circle*), 10 (*open circle*), 15 (*filled inverted triangle*), 20 (*open triangle*), 25 (*filled square*), and 30 μM (*open square*), respectively. **b** The IC_{50} value was obtained from a plot of percent tubulin polymerization against log concentration (M) of compound **3** at 37°C. *Inset*: column scatter graph of absorbance against compound **3** concentration in the reaction mixture. IC_{50} = 19.59 μM, log IC_{50} ± SE = −4.7 ± 0.0007

the inhibitory activity of these compounds was more potent than that of curcumin ($IC_{50} = 26.6 \pm 1.0$ μM).

Among these compounds, with the ability of tubulin assembly inhibition, compound **3** with IC_{50} value of 19.6 μM was selected for further studies including binding to tubulin and affecting its CD spectra. Figure 1 demonstrates that there is a concentration-dependent inhibition of microtubule assembly by 30 μM concentration of compound **3** with 89% inhibition. Based on transmission electron microscopy (TEM) micrographs, the length of microtubule filaments reduced in the presence of 20 and 30 μM of compound **3** as compared to the control experiment (Fig. 2).

Preliminary cytotoxicity evaluation

All of the synthesized compounds were evaluated for their inhibitory activity on the growth of K562 and SK-N-MC human cancer cell lines by measuring cell number in a concentration-dependent manner after 72 h of incubation. IC_{50} values were calculated from the semi-logarithmic dose–response plots using nonlinear regression program SigmaPlot (data not shown). The results have indicated that these compounds effectively inhibited the growth of K562 and SK-N-MC cancer cell lines. Generally, the inhibitory activities of all analogues against SK-N-MC cells were less significant than that of K562. Compound **4**, followed by compounds **3** and **5**, showed more potent cytotoxic activity in respect with natural compound curcumin (Table 1).

The binding of compound **3** to tubulin

As tubulin is the major component of microtubules, we wanted to test whether the effects of compound **3** on microtubule network and microtubule assembly are due to its binding to tubulin. Although, target compounds show weak fluorescence in PEM buffer (Fig. 3). Following incubation with purified tubulin, the experimental compounds showed fluorescence with maximum emission of 430 nm upon excitation at 371 nm. For example, incubating a 1:1 molar ratio of compound **3** with tubulin resulted in several-fold increase in the fluorescence intensity of compound **3**. The emission spectrum of compound **3** showed a blue-shift of 8 nm upon binding to tubulin, indicating that compound **3** binds to a hydrophobic region of tubulin (Fig. 3a). Figure 3b shows the titration curve of a constant amount of tubulin (2 μM), treated with various concentrations of compound **3**. The dissociation constant (K_d) of compound **3** to tubulin was calculated to be 3.7 ± 0.3 μM by the Scatchard plot using GraphPad Prism software (Fig. 3b, Inset). Also, the tryptophan residues in tubulin are intrinsically fluorescent and modulation of ligand-directed fluorescence of tryptophan residues, as determined by fluorescence spectroscopy has been extensively used to investigate tubulin-ligand binding. Compound **3** reduces the intrinsic fluorescence of tryptophan residue within the tubulin in a concentration-dependent manner, suggesting that it induces conformational change in tubulin (Fig. 4).

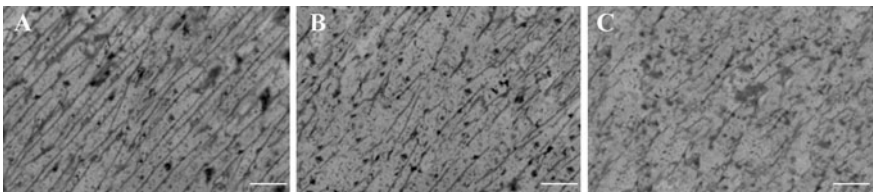


Fig. 2 TEM micrographs of microtubule polymers in the absence (**a**) and presence of 20 μM (**b**) and 30 μM (**c**) of compound **3**. *Scale bars*, 2 μm

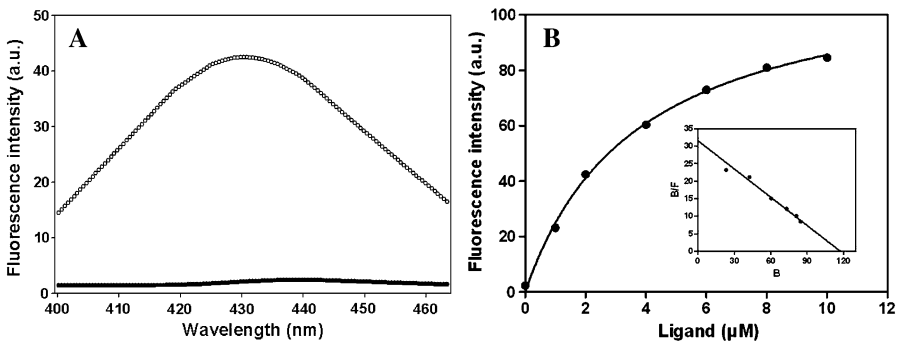


Fig. 3 Characterization of the binding of compound **3** with tubulin. **a** Change in fluorescence spectra of compound **3** after binding to tubulin. Compound **3** (2 μM) was incubated without (*filled square*), or with (*open circle*) tubulin (2 μM). The λ_{ex} and λ_{em} values were 371 and 423 nm, respectively. **b** Measurement of compound **3** binding to tubulin by fluorescence spectroscopy. 2 μM of tubulin was incubated with different concentrations of compound **3** (0–10 μM) and the fluorescence intensity was measured as described in the "Fluorescence measurements" section. *Inset* shows the scatchard plot of compound **3** binding to tubulin

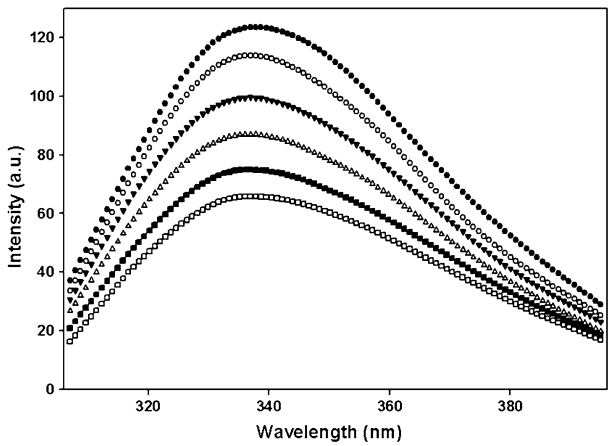


Fig. 4 Effects of compound **3** on the intrinsic tryptophan fluorescence of tubulin. Tubulin (2 μM) was incubated without (*filled circle*) or with 1 (*open circle*), 2 (*filled inverted triangle*), 4 (*open triangle*), 6 (*filled square*), and 8 (*open square*) μM of compound **3** for 30 min at 25°C. Compound **3** reduced the intrinsic fluorescence of tubulin with the excitation wavelength of 295 nm

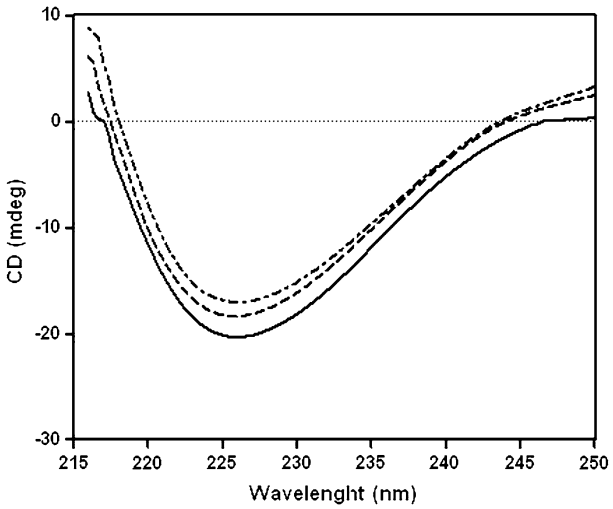


Fig. 5 Effect of compound **3** on the CD spectrum of tubulin. The graph shows the far-UV CD spectra of 4 μM tubulin in the absence (*solid line*) and presence of 25 (*dashed line*) and 50 μM (*dash-dotted line*) of compound **3** in a 10 mM phosphate buffer (pH 6.8). Each spectrum was the average of three scans

Effect of compound **3** on the Cd spectra of tubulin

Compound **3** inhibits the polymerization of microtubule proteins and decreases tryptophan fluorescence. The effect of compound **3** on the secondary structure of tubulin was further examined by circular dichroism spectroscopy. Compound **3** alters the far-UV CD spectrum of tubulin, indicating that ligand binding altered the helical content in tubulin (Fig. 5).

Discussion

During the last years, antimitotic agents have been used as chemotherapeutic agents to inhibit cell proliferation and induce cytotoxicity. The action mechanism of these agents often involves an interaction with microtubules, particularly tubulin. Microtubules are polar structures and are long protein fibers that exist in dynamic equilibrium with the tubulin dimer. Microtubules are vital components of the cell and are responsible for several important functions including intracellular transport, formation of the mitotic apparatus, mechanically stabilizing cellular processes, and formation of the mitotic spindle during cell division (Brown *et al.*, 2006). Antimitotic agents, including a diverse group of compounds are originally taken from natural sources (Jordan and Wilson, 2004). During the present study, we have investigated the anti-microtubule and anti-proliferative properties of 2′-hydroxychalcones as the synthetic analogues of curcumin. Curcumin is a natural product and possesses two methoxyphenolic rings, connected by two conjugated α,β-unsaturated ketones. Our target compounds are small molecules with aromatic ring A and B, linked by α,β-unsaturated ketone and easily prepared by convenient synthetic method. Chlorine substitution on the A and B rings was used for structural modification and modulation of basic pharmacophore of 2′-hydroxychalcones. Halogens, like chlorine, are very useful to modulate the electronic effects on the phenyl rings of drugs. Moreover, chlorine may also influence the steric characteristics and hydrophilic–hydrophobic balance of the molecules (Legraverend *et al.*, 2000; Zhang *et al.*, 2003). All chloro-substituted-2′-hydroxychalcones exhibit respectable tubulin inhibition and cytotoxic activities against two selected cancer cell lines (K562 and SK-N-MC). Their activities were more potent than that of curcumin. Such kind of biological activities are exhibited by other potent antimitotic compounds (Ducki *et al.*, 1998). Among these compounds, 3,5′-dichloro-analogue (compound **3**) with IC_{50} value of <20 μM in tubulin inhibition assay was selected for further studies. Microtubule assembly was significantly inhibited by compound **3** with the reduction of nucleation, polymerization rate, and maximum absorbance. Compound **3** inhibits tubulin polymerization with an IC_{50} of 19.6 μM, which is close to those exhibited by the compounds **5** (2,5′-dichloro- analogue) and 4 (4,5′-dichloro- analogue). By comparing the unsubstituted analogue (compound **1**) and chloro-substituted analogues (compounds **3**, **4**, and **5**), it has been observed that the addition of chloro-substituent to ring A/B causes an increase in hydrophobic interaction and biological activity of compounds. While, comparing the biological activities of compounds **4** (4,5′-dichloro- analogue) and **2**

(2,4,5′-trichloro- analogue), it can be concluded that the additional chloro-substitution (2-chloro) diminishes the biological activity of compound **2**, which may be due to the steric hindrance of the substitution. On the basis of significant inhibition of microtubules in vitro, we also examined the cytotoxic effects of our test compounds on K562 and SK-N-MC cancer cell lines and found that these compounds inhibit the cell proliferation with IC_{50} values higher than those of cell-free systems. These findings (based on the experiments of microtubule and cancer cell line) suggest that the weaker effects of these compounds, on the cells in comparison with microtubules, may be due to the presence of biological barrier such as cell membrane. Also, the inhibitory activities of all analogues against K562 are slightly more significant than those of SK-N-MC cells. Electron microscopic analysis of the microtubule polymers showed that compound **3** strongly reduced the length and extent of formation in microtubules. The decrease in polymer length and bundling of microtubules may be due to the binding of ligand molecules with tubulin. Compound **3** shows fluorescence upon binding to tubulin, although the drug itself does not have fluorescence in aqueous solution. The interaction of compound **3** with tubulin and microtubules indicates that our test compound binds to tubulin with a K_d of 3.7 ± 0.3 μM. In addition, compound **3** reduced the helical content, while increasing the random coil content of tubulin, suggesting that the perturbation in secondary structure of tubulin might also be the cause of destabilization of microtubules (Gupta and Panda, 2002).

In brief, the results suggest that chloro-substituted-2′-hydroxychalcones inhibit cell proliferation and tubulin assembly by inducing conformational changes in the protein. These findings may help in designing new synthetic analogues of curcumin with potent cytotoxic activity, improved stability, and bioavailability.

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