Colchicine-like β-acetamidoketones as inhibitors of microtubule polymerization: Design, synthesis and biological evaluation of in vitro anticancer activity

Ehsan Karimikia 1,2, Javad Behravan 1, Afsin Zarghi 3, Morteza Ghandadi 2, Sina Omid Malayeri 2, Razieh Ghodsi 1, 2*

1 Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
2 Department of Medicinal Chemistry, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
3 Department of Pharmaceutical Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

**Keywords:**
- Anticancer activity
- Chalcone
- Colchicine
- Colchicine-like
- Molecular docking
- Tubulin polymerization
- β-acetamidoketones

**ABSTRACT**

**Objective(s):** In this study a series of novel colchicine-like β-acetamidoketones was designed and synthesized as potential tubulin inhibitors.

**Materials and Methods:** The cytotoxicity of the novel synthesized β-acetamidoketones was assessed against two cancerous cell lines including MCF-7 (human breast cancer cells) and A549 (adenocarcinomic human alveolar basal epithelial cells) employing the MTT test. Tubulin polymerization test was done by using a commercial kit (Tubulin Polymerization Assay Kit).

**Results:** In general, the cytotoxicity activities were highly dependent on the aromatic substitution pattern of phenyl ring at β position of β-acetamidoketones. Based upon, compound 4f possessing the same structural elements of colchicine and chalcone 1, revealed the most cytotoxicity more than the other β-acetamidoketone against the cancerous cell lines and showed moderate antitubulin effect. The tubulin inhibitory effect of 4f, colchicine and chalcone 1 were consistent with their antiproliferative activities. Molecular docking studies of 4f into the colchicine-binding site of tubulin exhibited possible mode of interaction between this compound and tubulin.

**Conclusion:** The structure activity relationship (SAR) data attained showed that the presence of trimethoxy phenyl attached to carbonyl group of β-acetamidoketones and a methoxy group at para position of the other ring are essential for cytotoxic activity. In general, the cytotoxicity activities were highly dependent on the aromatic substitution pattern of phenyl ring at β position of β-acetamidoketones.

**Introduction**

Cancer is the reason of one-quarter of all expiries in industrialized countries. Nowadays cancer is the second reason of death in the United States, and is predicted to exceed heart illnesses as the leading cause of death in the next years (1). Therefore; the discovery of anticancer, non-toxic and drug-like active compounds is still an urgent demand.

Microtubules play key role in mitosis and are imperative target for the design of novel anticancer agents. Colchicine (Figure 1) a compound isolated from *Colchicum autumnale* and *Gloriosa superba* was the first drug identified to bind tubulin, and it binds at a specific site called the colchicine domain (2). There are various compounds discovered, which bind tubulins at the colchicine binding site, and hence by inhibiting microtubule polymerization cause cell cycle arrest and lead to cell death. (3). Combretastatins, separated from the South African tree *Combretum caffrum*, are a series of antimitotic agents. Combretastatin A-4 (CA-4, Figure 1) is a natural anti-tubulin compound which binds to colchicine’s binding site of tubulin and exerts its antimitotic effect. Many structural modifications of the combretastatin A-4 molecule, such as variation of substitutions on the A- and B-rings, have been reported (4-6). Numerous Chalcones are also reported as tubulin inhibitors (7, 8). Chalcone 1, a combretastatin-like derivative was known to possess potent anticancer activity and tubulin inhibition effect (7, 9).

Colchicine is identified to have significant anti-mitotic, anti-inflammatory and anti-fibrotic effects. Although colchicine possesses notable in vitro antitumor effects, its therapeutic uses have been restricted because of the low bioavailability and high toxicity (10). Though it’s unfavorable pharmacological profiles, colchicine is still a lead compound for the discovering of possible antimitotic drugs. Thus, various analogs of colchicine (11-14) have been synthesized with the goal of discovering novel, valuable drugs with more bioavailability and favorable pharmacological effects. Structure-activity studies (15, 16) suggested that the trimethoxy phenyl ring (A) and the methoxy tropone ring (C) of colchicine include the minimal structural features of the compound needed for its binding to tubulin. On the other hand, a study (17) revealed that the B-ring of colchicine plays a key role in the stability of tubulin binding while the A and the
C-rings have not significant effect on it and colchicine binds at the α/β interface of tubulin. The B-ring binds on the α-subunit and the A and the C-rings bind on the β-subunit.

In the present study we report the design and synthesis of novel β-acetamidoketones possessing pharmacophoric requirements of anti-tubulins with central β-acetamido ketone bridge (Figure 1). They designed to bear some similarity to colchicine and chalcones. We designed the hybrid compounds, combining ring A and a part of ring B of colchicine and a fragment of chalcone 1 with the same linker length. (Figure 1). They were evaluated for their antiproliferative properties against two human cancer cell lines including MCF-7 and A549. The tubulin inhibitory effect of 4f (the most cytotoxic compound) was also evaluated. In order to get the better structure activity relationship (SAR) data, we also evaluated the cytotoxicity and tubulin inhibitory properties of chalcone 1.

**Experimental**

All reagents, chemicals and solvents used in the present study were bought from Merck AG and Aldrich Chemical. Melting points were measured using a Thomas-Hoover capillary apparatus. Infrared spectra were attained by a Perkin Elmer Model 1420 spectrometer (Germany). 1H NMR and 13C NMR spectra were attained by Bruker FT-300 MHz instrument (Brucker Biosciences, USA). Chloroform-D was used as solvent. Coupling constant (J) values were assessed in hertz (Hz) and spin multiples were given as s (singlet), d (double), t (triplet), q (quartet), m (multiplet). The mass spectra were acquired using a 6410 Agilent LCMS triple quadrupole mass spectrometer (LCMS) with an electrospray ionization (ESI) interface (Japan). Elemental analyses were done on a Cos-Tec model EAS 4010 instrument (Gernusco, Italy) and the results were within ±0.4% of the theoretical values.

**General procedure for the synthesis of β-acetamido propiophenones**

Substituted benzaldehyde (1.0 mmol) and acetyl chloride (0.5 ml) in acetonitrile (3.0 ml), TFA (0.30 mmole%) were added to a solution of an appropriate acetophenone (1.0 mmol), and the mixture was stirred for about 30 min at an ice-water bath, and then permitted to warm to room temperature. Upon completion of the reaction, the mixture was transferred into ice-water (20.0 ml) saturated NaHCO3 was added to the mixture to adjust the pH to 7, which resulted to precipitation of the target β-acetamidoketone. The precipitate was filtered, washed with hexane and recrystallized in methanol.

**β-acetamido-β-(phenyl)-3,4,5-trimethoxy propiophenone (4a)**

Yield, 69%; mp= 101-103 °C: IR (KBr) ν (cm⁻¹): 1658 (C=O), 3266 (NH) ; 1H NMR (300 MHz) (CDCl3) δ (ppm): 1.67 (s, 3H, CH3), 3.35-3.43(dd, 1H, CH2, J=6.6 & 16.2 Hz), 3.78-3.85 (dd, 1H, CH2, J=6.6 & 16.2 Hz), 3.88 (s, 6H, OCH3), 3.90 (s, 3H, OCH3), 5.53-5.59 (m, 1H, CH), 6.61-6.63 (d, 1H, NH, J=7.5), 7.21 (s, 2H, 3,4,5-trimethoxyphenyl H2 & H6), 7.25-7.36 (m, 5H, phenyl): 13C NMR (75 MHz) (CDCl3) δ (ppm): 23.38, 43.34, 50.48, 56.29, 60.94, 105.69, 126.58, 127.57, 128.71, 131.79, 140.78, 142.85, 153.07, 169.57, 197.14; MS (ESI) m/z:358.2 [M+1], 380.2 [M+23]. Anal. Calcd for C20H23NO5: C, 67.21; H, 6.49; N, 3.92. Found: C, 67.43; H, 6.31; N, 4.12.

**β-acetamido-β-(4-methylphenyl)-3,4,5-trimethoxy propiophenone (4b)**

Yield, 69%; mp= 118-120°C: IR (KBr) ν (cm⁻¹): 1647 (C=O), 3319 (NH) ; 1H NMR (300 MHz) (CDCl3) δ (ppm): 2.05 (s, 3H, CH3), 2.33 (s, 3H, CH3), 3.32-3.34 (dd, 1H, CH2, J=6.9 &16.2 Hz), 3.77-3.84 (dd, 1H, CH2, J=6.9 &16.2 Hz), 3.77 (s, 6H, OCH3), 3.79 (s, 3H, OCH3), 5.50-5.52 (m, 1H, CH), 6.51-6.53 (d, 1H, NH, J=7.5), 7.14-7.16 (d, 2H, 4-methylphenyl H2 & H6), 7.22-7.25 (m, 4H, H7-10).
Yield, 69%; mp= 155-157°C: IR (KBr) ν (cm⁻¹): 1617, 1774 (C=O), 3240 (NH). 1H NMR (300 MHz) (CDCl₃) δ (ppm): 2.01 (s, 3H, CH₃), 2.28 (s, 3H, CH₃), 3.31-3.35 (dd, 1H, CH₃, J=6.9 & 16.4 Hz), 3.74-3.79 (dd, 1H, CH₃, J=6.9 & 16.4 Hz), 3.80 (s, 3H, CH₂O), 3.88-4.04 (m, 9H, OCH₃), 5.44-5.48 (m, 1H, CH), 6.57 (s, 1H, NH), 6.88-6.90 (d, 1H, 4-acetoxy-3-methoxyphenyl H, J=8.51), 7.02-7.03 (d, 1H, 4-acetoxy-3-methoxyphenyl H, J=2.1 Hz), 7.15-7.17 (dd, 1H, 4-acetoxy-3-methoxyphenyl H, J=2.1 & 8.51 Hz), 7.20 (s, 2H, 3,4,5-trimethoxyphenyl H, J=7.2 & 16.2 Hz). 13C NMR (75 MHz) (CDCl₃) δ (ppm): 20.66, 23.43, 40.48, 49.78, 55.94, 56.33, 60.95, 105.72, 112.40, 121.40, 125.21, 131.71, 133.24, 139.75, 142.92, 150.48, 153.12, 168.93, 169.50, 197.05 (MS) (EI) m/z: 446.2 [M+1]+, 468.2 [M+2]. Anal. Calcd for C₂₃H₂₅NO₅: C, 67.91; H, 6.78; N, 3.37. Found: C, 67.55; H, 6.91; N, 3.27.
δ (ppm): 20.65, 23.40, 42.73, 49.33, 55.51, 55.93, 111.82, 112.33, 113.87, 121.29, 125.04, 129.70, 130.52, 133.88, 139.70, 150.29, 163.83, 169.45, 196.93; MS (ESI) m/z:408.2 [M+23]. Anal. Calcd for C_{15}H_{16}NO_{5}: C, 65.56; H, 6.36; N, 3.39.

β-acetamido-β(3-acetoxy, 4-methoxyphenyl)-4-fluoro propiophenone (4f)

Yield, 85%; mp= 186-188 °C; IR (KBr) v (cm⁻¹): 1618, 1690 (C=O), 3416 (OH); ^1H NMR (300 MHz) (CDCl₃) δ (ppm): 2.05 (s, 3H, CH₃), 3.28-3.36 (dd, 1H, CH₃=J=6.9 & 15.9 Hz), 3.74-3.81(dd,1H, CH₃=J=6.9 & 15.9 Hz), 3.87 (s, 3H, OCH₃), 3.88-4.01 (m, 9H, OCH₃), 5.45-5.46 (m, 1H, CH), 5.73 (1H, OH), 6.51-6.53 (d, 1H, NH, J=6.9), 6.80-6.92(m, 3H,3-hydroxy-4-methoxyphenyl H, H, & H); ^13CNMR (75 MHz) δ (ppm): 23.40, 43.33, 50.63, 60.14, 70.77, 103.88, 115.74, 116.03, 130.80, 130.93, 133.11, 135.57, 153.34, 169.62, 197.04: MS (ESI) m/z: 376.2[M+1], 398.1 [M+23]. Anal. Calcd for C_{19}H_{25}NO_{5}: C, 66.58; H, 6.58; N, 5.94.

Cytotoxicity assay

General procedure

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] based assay was performed by seeding 5000 cancerous cells per 180 µl RPMI complete culture medium in each well of 96-well culture plates (1822). After 24 hr, culture medium was substituted with medium having positive control colchicine and chalcone 1 as well as diverse concentrations of new synthesized compounds and RPMI as negative control. Then cells were incubated at 37 °C in 5% CO₂ incubator for 48 hr. Then 25 µl of MTT solution (4 mg ml⁻¹) were added to each well and further incubated at 37 °C for 3 hr, then formazan crystals were dissolved in DMSO (100 µl) and plates were read using a plate reader (Synergy H4, USA) at 540 nm. This test was done in triplelicate determination each time.

Tubulin polymerization assay

Tubulin polymerization test was done by using a commercial kit (Tubulin Polymerization Assay Kit (Porcine tubulin and Fluorescence based Kit, Cat No BK011P, Cytoskeleton, USA), based on the manufacturer's procedure (23-25). Tubulin protein was added to tubulin buffer (80 mM PIPES, 2 mM MgCl₂, 0.5 mM EGTA, 1 mM GTP, 60% (v/v) glycerol, pH 6.9) then poured to wells of a 96-well plate possessing the cytoxic chemicals or vehicle and then mixed well. The effects of compound 4f and Chalcone 1 at 50 and 100 µM concentrations on tubulin polymerization were investigated.

Tubulin polymerization was monitored by detecting the enhancement of the fluorescence because of the addition of a fluorescence reporter into microtubules as polymerization occurs. Polymerization was measured by recording at 360 nm and emission at 420 nm for...
Novel β-acetamidoketones as tubulin inhibitors
Karimikia et al.

Molecular modeling

Mode of interaction between 4f and tubulin was evaluated by docking. 2D structure of the compound was organized in Chem Draw Ultra 8.0 software and 3D structure was prepared by Hyperchem 7 software via molecular mechanic force filed pre-optimization followed by AM1 semiempirical calculation. The X-ray crystal structure of tubulin (PDB ID: 1SA0) was copied from the Protein Data Bank (www.rcsb.org). Further changes such as polar hydrogen adding and water molecules deletion was done by MOE software. Compound 4f was docked into the binding site of tubulin using MOE software. Every atom in a 5 Å about the co-crystallized ligand in crystal coordinates of tubulin was selected as binding site. The docking simulations were performed using triangle matcher placement algorithm in combination with London dG scoring function and force field as refinement process. The top-score docking poses were selected for final ligand–target interaction analysis using LigX module in MOE Software. Validation of docking method was first assessed by docking of co-crystallized ligand into the tubulin binding site (26).

Results

Synthesis

As depicted in scheme 1, In order to synthesize 4f, at first we let 3,4,5-trimethoxy acetophenone 2a and 3-hydroxy-4-methoxy benzaldehyde 3e condense in acetonitrile in the presence of catalytic amount of Cerium (IV) sulfate according to the reported procedure (27) but no reaction was happened. To get the target 4f, we examined another reported method (28) employing boric acid as a catalyst and acetyl chloride in acetonitrile. Interestingly, the IR and 1H-NMR spectra revealed that the chalcone 1 was formed instead. Finally, we tried to do the reaction using trifluoroacetic acid as a catalyst in the presence of acetoxy chloride in acetonitrile (29). The presence of a peak in 1758 cm⁻¹ in the IR spectrum and also a singlet peak in 2.31 ppm in 1HNMR spectrum proved the formation of 4e which possesses the acetoxy group instead of hydroxyl group. Hydrolysis of acetoxy group of 4e in the presence of potassium carbonate in NMP (N-methyl-2-pyrrolidone) at 100 °C (28) led to the formation of 4f. We found that TFA is the best catalyst for synthesis of our β-acetamidoketones (Scheme 2), and acetylation of hydroxyl group was observed while using substituted benzaldehyde bearing hydroxyl group (3d, 3e and 3f). The compounds were characterized by nuclear magnetic resonance, infrared, mass spectrometry and elementary analysis.

Biological evaluation

In vitro anticancer activity

The cytotoxicity of the synthesized compounds was assessed against two cancerous cell lines including MCF-7 (human breast cancer cells) and A549 (adenocarcinomic human alveolar basal epithelial cells) using the MTT test. As shown in Table 1, by comparing the cytotoxicity of 4j and 4k (which displayed no activity at the concentrations below 100 µM), with those of 4e and 4f, we can conclude that trimethoxy phenyl moiety attached to carbonyl group is essential for cytotoxic activity of these compounds. The cytotoxicity activity was highly dependent on the aromatic substitution pattern of phenyl ring at β position of β-acetamidoketones. SAR data showed that, introducing an electron donating group in the para position of 4a increased its cytotoxic activity against MCF-7 cells (see the anti-proliferative activities of 4a-4d in Table 1). According to our results, compound 4f was at least twice more cytotoxic than acetoxy analogue 4e. Comparing the cytotoxic activity

Scheme 1. Reagents and conditions: (a) Cerium (IV) sulfate, CH₃CN (b) H₃BO₃, CH₃CN, CH₃COCl (c) TFA, CH₃CN, CH₃COCl (d) K₂CO₃, NMP
of 4f with its parent compound 4e shows that the hydroxyl group at meta position of phenyl ring has a crucial role for its activity which may explain the ability of hydroxyl group to form hydrogen bond within the active site of tubulin as a hydrogen binding donor. Replacement of the acetamido group at para position of phenyl ring (4c) by acetoxy substituent (4d) increased cytotoxic activity considerably. Our results indicated that compound 4i possessing trimethoxy phenyl ring at β position showed more cytotoxicity activities than 4h having dimethoxy phenyl ring in MCF-7 cells. According to our results, compound 4f possessing the colchicine and chalcone 1 aromatic substitution pattern showed the highest cytotoxicity among the β-acetamidoketones series against the cancer cell lines. As chalcone 1 showed higher anti-proliferative activity compared to colchicine, it can be concluded that α, β-unsaturated bridge of chalcone 1 might act as the Michael acceptor in tubulin binding site or other targets in addition to rigid linkage. In general, our compounds exhibited more cytotoxicity effects in MCF-7 cancer cells compared to A549 cancer cells, demonstrating that the compounds may exert their cytotoxicity with different mechanisms in different cancer cells.

**Tubulin polymerization assay**

In order to clarify whether the cytotoxic activity of 4f was related to the tubulin binding ability, compounds 4f (the most cytotoxic compound), chalcone 1 and reference compound colchicine (polymerization suppressor) and a polymerization promoter (paclitaxel) were assessed for its effect on tubulin polymerization.

![Scheme 2. Reagents and conditions: (a) TFA, CH₃CN, CH₃COCl (b) K₂CO₃, NMP, 100 °C](image_url)

**Table 1.** The in vitro anti-proliferative activities of colchicine-like β-acetamidoketones, colchicine, and chalcone 1 against human cancer cell lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>R₅</th>
<th>R₆</th>
<th>IC₅₀ (µM) MCF-7</th>
<th>IC₅₀ (µM) A549</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4b</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>H</td>
<td>CH₃</td>
<td>H</td>
<td>41.9 ± 4.72</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4c</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
<td>69.7 ± 9.1</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4d</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>H</td>
<td>OOCCH₃</td>
<td>H</td>
<td>30.4 ± 2.78</td>
<td>48.9 ± 3.98</td>
</tr>
<tr>
<td>4e</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>H</td>
<td>34.1 ± 3.5</td>
<td>48.8 ± 4.2</td>
</tr>
<tr>
<td>4f</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OH</td>
<td>OCH₃</td>
<td>H</td>
<td>14.6 ± 2.1</td>
<td>23.2 ± 1.95</td>
</tr>
<tr>
<td>4g</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>H</td>
<td>36.2 ± 3.5</td>
<td>49.3 ± 3.98</td>
</tr>
<tr>
<td>4h</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>H</td>
<td>37.2 ± 4.12</td>
<td>37.7 ± 3.05</td>
</tr>
<tr>
<td>4i</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>18.8 ± 1.1</td>
<td>40.8 ± 2.7</td>
</tr>
<tr>
<td>4j</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
<td>OOCCH₃</td>
<td>OCH₃</td>
<td>H</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4k</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
<td>OH</td>
<td>OCH₃</td>
<td>H</td>
<td>&gt;100</td>
<td>87 ± 7.5</td>
</tr>
<tr>
<td>4l</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>OOCCH₃</td>
<td>OCH₃</td>
<td>H</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4m</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Chalcone 1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.9 ± 0.27</td>
<td>2.9 ± 0.43</td>
</tr>
<tr>
<td>Colchicine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.1 ± 0.37</td>
<td>ND</td>
</tr>
</tbody>
</table>
As illustrated in Figure 2, 4f proved to be inhibitor of tubulin polymerization in a manner similar to that of chalcone 1 and colchicine. However, 4f was not as potent as these reference tubulin inhibitors but its tubulin inhibition effect was dose dependent. SAR acquired data indicated that flexible bridge in 4f could lead to conformational flexibility that is unfavorable for tubulin inhibition. Because of higher tubulin inhibitory effect of chalcone 1 compared with colchicine it can be concluded that α, β-unsaturated bridge of chalcone might act as the Michael acceptor in tubulin binding site in addition to the rigid linkage. The tubulin inhibitory effect of 4f, colchicine and chalcone 1 were consistent with their antiproliferative activities. On the other hand, the order of tubulin inhibitory effect of these three compounds is the same as their antiproliferative activities.

**Molecular modeling (docking) studies**

In an attempt to elucidate the possible binding mode of this novel series of tubulin inhibitors, docking of the most potent compound 4f was performed at the active site of the tubulin dimer. The quality and validity of docking procedure were investigated by docking of co-crystallized ligand within the binding site of tubulin. From docking studies, the top binding position exhibited a similar alignment in the binding pocket to the co-crystallized ligand found in crystal assembly (PDB ID of 1SA0). The root mean square deviation (RMSD) between co-crystallized ligand within the binding site and ligand docked in the crystal structure of tubulin was 1.4 Å, indicative of a proper ability to mimic the ligand binding mode recognized in the experimental data (30, 31). As mentioned above, compound 4f possessing structural elements of colchicine and chalcone 1, demonstrated the most anti proliferative activity compared to the other synthesized compounds which inhibited the polymerization of tubulin. According to ligand interaction mode of 4f by LigX module of MOE software, hydroxyl group of 4f could form hydrogen bonds with LysB241, ValB318, AlaB316, AlaB317 and LysB352. The β-acetamido side chain of 4f was surrounded by residues AlaB354, AlaA180, SerA178, ThrB353, ThrA179 and AsnB249 and could make contacts with them. These hydrophobic interactions and hydrogen bond formation of 4f with tubulin binding site can explain inhibitory effect of this compound.

**Discussion**

A new series of colchicine-like β-acetamidoketone analogues was synthesized and evaluated for their cytotoxic activity against MCF-7 and A549 cancer cells. The structure activity data acquired indicate that the presence of trimethoxy phenyl attached to carbonyl group of and a methoxy group at para position of the other ring in the synthesized compounds are essential for cytotoxic activity. As colchicine 1 showed higher antiproliferative activity compared to chalcone 1, it can be concluded that α, β-unsaturated bridge of chalcone 1 might act as the Michael acceptor in tubulin binding site or other targets in addition to rigid linkage. In general, the cytotoxic activities were highly dependent on the aromatic substitution pattern of the phenyl ring at position β of β-acetamidoketones. Based upon, compound 4f possessing the trimethoxyphenyl and 3-hydroxy-4-methoxy groups, demonstrated the best cytotoxicity among the other β-acetamidoketones against the cancerous cell lines and proved to be a tubulin inhibitor. Finally, molecular docking studies of 4f into the colchicine-binding site of tubulin demonstrated the possible interactions of this compound at the active site of tubulin. The hydrophobic interactions and hydrogen bonds formation of 4f with tubulin binding site can explain inhibitory effect of this compound.

**Conclusion**

The cytotoxicity activities were highly dependent on the aromatic substitution pattern of phenyl ring at position β of β-acetamidoketones. Based upon, compound 4f possessing the same structural elements of colchicine and chalcone 1, revealed the most
cytotoxicity more than the other β-acetamidoketone against the cancerous cell lines and showed moderate antitubulin effect. The tubulin inhibitory effect of 4f, colchicine and chalcone 1 were consistent with their anti-proliferative effects. Molecular docking studies of 4f, into the colchicine-binding site of tubulin exhibited possible mode of interaction between this compound and tubulin.

Acknowledgment

We are grateful to Research Deputy of Mashhad University of Medical Sciences, Mashhad, Iran for financial support of this research as part of thesis of Ehsan Karimikia.

Conflicts of Interest

The authors declare no conflict of interest.

References

30. Chalraborti A, Sharma L, Sharma U. A mild and