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RESEARCH ARTICLE

Synthesis and cytotoxic activity of certain trisubstituted azetidin-2-one derivatives as a *cis*-restricted combretastatin A-4 analogues

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Abstract Novel series of 1,3,4-trisubstituted azetidin-2-one derivatives **8a–p** were synthesized and proposed as cytotoxic agents acting via inhibition of tubulin at the colchicine binding site. The design of the target compounds was based upon modification in the structure of the vascular targeting agent combretastatin A-4 (CA-4). The *cis* double bond linker in CA-4 was replaced with the azetidin-2-one ring aiming to prevent the *cis/trans* isomerization that suppresses the activity of CA-4, thereby enhancing its antiproliferative activity. All new compounds were investigated in vitro against MCF-7 and HCT-116 cell lines. The inhibition of tubulin polymerization by four most potent compounds **8g**, **8j**, **8n** and **8o** was also evaluated. The synthesis of the final targets was achieved adopting Staudinger reaction. Molecular modeling studies were performed to rationalize the biological results.

Keywords Tubulin · Combretastatin A4 · Azetidin-2-one · Cytotoxic activity

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Introduction

Antimitotic agents comprise one of the major classes in chemotherapy, and microtubules are a significant target for many natural anticancer agents (Jordan 2002). Microtubules are the main constituents of the mitotic spindle and are formed by association of protofilaments that are made up of alternating α and β tubulin monomers. They are involved in many essential functions throughout the cell cycle including transport of material within the cell, movement of the cell itself, and proper progression through cell division (Downing 2000). Drugs acting as tubulin inhibitors arrest the cell during mitosis, as they have the ability to disrupt microtubule assembly or disassembly making them preferential for tumor cells with high proliferation rate (Dumontet and Jordan 2010).

Combretastatins are a group of tubulin-binding diarylstilbenes isolated from the South African tree *Combretum caffrum*. Combretastatin-A4 (CA-4) **2** comprises as one of the most powerful antimitotic agents that inhibits the formation of mitotic spindle through binding to tubulin at the colchicine binding site, thereby interfering with cell growth and proliferation (Dumontet and Jordan 2010; Cragg and Newman 2005; Lippert 2007). In contrast to colchicine **1**, the anti-vascular effects of CA-4 in vivo are apparent well below the maximum tolerated dose, offering a wide therapeutic window (Tozer et al. 1999; West and Price 2004). A water soluble pro-drug combretastatin A-4 phosphate (CA-4P) **3** is under evaluation in phase 2 clinical trials for treatment of non-small cell lung cancer and in phase 3 for treatment of anaplastic thyroid cancer (O'Boyle et al. 2011a).

Many conformationally restricted analogues of CA-4 have been reported (Wang et al. 2002; Ohsumi et al. 1998), in which the isomerizable *cis*-double bond is replaced with a heterocyclic ring, among which, the azetidin-2-one (β-

lactam) ring comprises one of the important scaffolds (O'Boyle et al. 2011b). The anticancer activity of some β-lactam-containing compounds including potent non-isomerizable CA-4 analogues have been reported (Banik et al. 2003).

Herein, it was of interest to synthesize certain new combretastatin-related compounds containing the β-lactam ring with the aim to provide rigidification of the structure to prevent *cis*/*trans* isomerization observed in CA-4. The β-lactam ring was also supposed to impart a useful scaffold structure with a similar spatial arrangement between the two phenyl rings as observed in the *cis* conformation of CA-4. Moreover, in the newly synthesized compounds the trimethoxyphenyl scaffold which is present in colchicine and CA-4 was retained at *N*-1 or at C-4 of the azetidine-2-one ring, that could afford additional sites of interaction with the colchicine binding site.

Materials and methods

Chemistry

The starting materials **6a–e** and **6g–i** were prepared as reported. Other chemicals and reagents were obtained from Aldrich, Fluca or Merck and were used without further purification unless otherwise indicated. Methylene chloride was dried by distillation from calcium hydride prior to use. Progress of the reactions was monitored using TLC sheets precoated with UV fluorescent silica gel Merck 60F 254. The solvent system was hexane and ethyl acetate (8:2) and spots were visualized using UV lamp. IR spectra were determined on Shimadzu FT-IR 8400 s spectrophotometer (KBr, cm^{-1}). ^1H NMR spectra were carried out using a Mercury 300-BB 300 MHz and Bruker 400 MHz spectrophotometers using tetramethylsilane (TMS) as internal standard. ^{13}C NMR spectra were carried out using a Mercury 300-BB 75 MHz and Bruker 100 MHz using TMS as internal standard. Chemical shifts (δ) are recorded in ppm on δ scale, Microanalytical unit, Faculty of Pharmacy, Cairo University, Egypt. Mass spectra were performed on Hewlett Packard 5988 spectrometer, Microanalytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt. Melting points were determined on Stuart apparatus and the values given are uncorrected. Column chromatography was carried out using standard silica gel 60 (230–400 mesh) obtained from Fluca.

*General method for preparation of Schiff bases (**6a–i**)*

A solution containing equimolar amounts of the appropriate substituted primary aromatic amine **4** and an aromatic aldehyde **5** (100 mmol each) in ethanol (50 mL) containing glacial acetic acid (2–3 drops) was heated under reflux for 5–12 h. After cooling, the crystalline solid product was filtered off, then recrystallized from ethanol. Compounds **6a–e** and **6g–i** were prepared and purified as previously reported (Al-Tai et al. 1976; Suresh et al. 2013; O'Boyle et al. 2010; Klimczak et al. 2012; Greene et al. 2016).

*N-(2-Bromobenzylidene)-3,4,5-trimethoxybenzenamine (**6f**)* Was prepared from 3,4,5-trimethoxybenzenamine and 2-bromobenzaldhyde applying the above procedure. Time of reflux 10 h, Yield 83 %, pale yellow crystals, mp 135–136 °C. IR (KBr, υ cm^{-1}): 3061 (CH aromatic), 2999, 2938 (CH aliphatic), 1584 (C=N); ^1H NMR (DMSO-d_6)δ ppm: 3.67 (s, 3H, OCH_3), 3.82 (s, 6H, $2OCH_3$), 6.63 (s, 2H, Ar–H), 7.44–7.54 (m, 2H, Ar–H), 7.76 (d, 1H, Ar–H, $J = 7.5$ Hz), 8.09 (d, 1H, Ar–H, $J = 7.5$ Hz), 8.78 (s, 1H, CH=N). EIMS m/z (%rel. abundance): 349.00 (M−H, 99.98), 350.00 (M^+, 29.59), 351.00 (M+H, 100), 352.00 (M+2, 27.63); Anal. Calcd for $C_{16}H_{16}BrNO_3$: C 54.87, H 4.61, N 4.00. Found: C 55.12, H 4.70, N 4.18.

*General method for preparation of **8a–p***

A mixture of the appropriate $\mathbf{6_{a-i}}$ (5 mmol) in anhydrous CH_2Cl_2 (50 mL) and TEA (15 mmol, 1.08 mL) was heated to reflux. A respective substituted acetyl chloride **7** (7.5 mmol) was injected drop wise through a rubber stopper over a period of 20 min, then heating was continued for additional 7–12 h. After cooling, the reaction mixture was washed with distilled water (50 mL × 2) followed by saturated aqueous $NaHCO_3$ solution (50 mL), then with 10 % brine (50 mL). The organic layer was dried over (anhyd. Na_2SO_4), and the solvent was distilled off under reduced pressure to give the crude product, which was further purified using column chromatography over silica gel 60 Å (70–230 mesh) and (hexane/ethyl acetate gradient) as an eluent.

*Microwave method for the preparation of **8l–o***

To a solution of the appropriate **6g–i** (5 mmol) in chlorobenzene was added a respective acid chloride **7** (7.5 mmol) and TEA (15 mmol, 1.08 mL). The mixture was irradiated in Milestone- StartSYNTH microwave oven for 10 min at 80 °C, then washed with distilled water (50 mL), saturated aqueous $NaHCO_3$ solution (50 mL) and brine (50 mL). The organic layer was dried (anhyd. Na_2SO_4), and concentrated under reduced pressure to give the crude product, which was further purified using column chromatography over silica gel 60Å (70–230 mesh) and (hexane/ethyl acetate gradient) as an eluent.

4-(2-Bromophenyl)-3-chloro-1-(4-methoxyphenyl)azetidin-2-one ***(8a)*** Was prepared from *N*-(2-bromobenzylidene)-4-methoxybenzenamine (**6a**) and acyl chloride **7**. Yield 48 %, buff crystals, mp 109–110 °C. IR (KBr, υ cm^{-1}): 3062 (CH aromatic), 2945, 2829 (CH aliphatic), 1761 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 3.71 (s, 3H, OCH_3), 5.23 (d, 1H, H-4, $J = 1.8$ Hz), 5.57 (d, 1H, H-3, $J = 1.8$ Hz), 6.94 (d, 2H, Ar–H, $J = 9$ Hz), 7.20 (d, 2H, Ar–H, $J = 9$ Hz), 7.23 (d, 1H, Ar–H, $J = 9.6$ Hz), 7.31–7.38 (m, 2H, Ar–H), 7.75 (d, 1H, Ar–H, $J = 9.6$ Hz); ^{13}C NMR (100 MHz, DMSO)δ ppm: 55.76 (OCH_3), 62.42 (C-3), 64.21 (C-4), 115.14 (2C), 119.05, 119.44 (2C), 128.35, 129.95 (2C), 131.26, 133.82, 134.32, 156.87 (ArC), 160.34 (C=O); EIMS m/z (%rel. abundance): 365.00 (M^+, 84.52), 367.00 (M+2, 80.95); Anal. Calcd for $C_{16}H_{13}BrClNO_2$: C 52.41, H 3.57, N 3.82. Found: C 52.51, H 3.60, N 3.89.

4-(2-Bromophenyl)-3-chloro-1-(4-methoxyphenyl)-3-methylazetidin-2-one ***(8b)*** Was prepared from *N*-(2-bromobenzylidene)-4-methoxybenzenamine (**6a**) and acyl chloride **7**. Yield 78 %, white crystals, mp 144–145 °C. IR (KBr, υ cm^{-1}): 3070 (CH aromatic), 2970, 2841 (CH aliphatic), 1761 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 2.11 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 5.74 (s, 1H, H-4), 6.96 (d, 2H, Ar–H, $J = 9$ Hz), 7.24 (d, 2H, Ar–H, $J = 9$ Hz), 7.30–7.36 (m, 3H, Ar–H), 7.76 (d, 1H, Ar–H, $J = 9$ Hz); ^{13}C NMR (100 MHz, DMSO)δ ppm: 24.88 (CH_3), 55.79 (OCH_3), 66.90 (C-3), 74.25 (C-4), 115.08 (2C), 115.13 (2C), 119.44, 119.71, 123.44, 128.20, 128.57, 130.07, 134.31, 156.84 (ArC), 163.28 (C=O); EIMS m/z (%rel. abundance): 379.87 (M^+, 2.87), 381.87 (M+2, 3.55), 383.87 (M+4, 0.87); Anal. Calcd for $C_{17}H_{15}BrClNO_2$: C 53.64, H 3.97, N, 3.68. Found: C 53.78, H 3.99, N 3.68.

4-(4-Bromophenyl)-3-chloro-1-(4-methoxyphenyl)azetidin-2-one ***(8c)*** Was prepared from *N*-(4-bromobenzylidene)-4-methoxybenzenamine (**6b**) and acyl chloride **7**. Yield 45 %, white rod crystals, mp 148–149 °C. IR (KBr, υ cm^{-1}): 3010 (CH aromatic), 2966, 2953 (CH aliphatic), 1751 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 3.69 (s, 3H, OCH_3), 5.21 (d, 1H, H-4, $J = 2.1$ Hz), 5.37 (d, 1H, H-3, $J = 2.1$), 6.88 (d, 2H, Ar–H, $J = 9$ Hz), 7.20 (d, 2H, Ar–H, $J = 9$ Hz), 7.42 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.60 (d, 2H, Ar–H, $J = 8.4$ Hz); ^{13}C NMR (100 MHz, DMSO)δ ppm: 55.74 (OCH_3), 62.61 (C-3), 64.19 (C-4), 115.06 (2C), 119.54, 122.90 (2C), 129.67 (2C), 129.76 (2C), 132.47, 134.91, 156.81 (ArC), 160.42 (C=O); EIMS m/z (%rel. abundance): 364.84 (M^+, 2.61), 366.85 (M+2, 3.51), 368.86 (M+4, 0.90); Anal. Calcd for $C_{16}H_{13}BrClNO_2$: C 52.41, H 3.57, N 3.82. Found: C 52.49, H 3.61, N 3.90.

3-Chloro-4-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-methylazetidin-2-one ***(8d)*** Was prepared from *N*-(4-chlorobenzylidene)-4-methoxybenzenamine (**6c**) and acyl chloride **7**. Yield 74 %, pale brown crystals, mp 108–109 °C. IR (KBr, υ cm^{-1}): 3028 (CH aromatic), 2987, 2839 (CH aliphatic), 1772 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 1.29 (s, 3H, CH_3), 3.70 (s, 3H, OCH_3), 5.63 (s, 1H, H-4), 6.91 (d, 2H, Ar–H, $J = 9$ Hz), 7.20 (d, 2H, Ar–H, $J = 9$ Hz), 7.36 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.46 (d, 2H, Ar–H, $J = 8.4$ Hz); ^{13}C NMR (100 MHz, DMSO)δ ppm: 20.76 (CH_3), 55.72 (OCH_3), 68.82 (C-3), 73.24 (C-4), 115.06 (2C), 119.66 (2C), 122.98 (2C), 129.34 (2C), 129.63, 132.36, 134.13, 156.82 (ArC), 162.71 (C=O); EIMS m/z (%rel. abundance): 335.00 (M^+, 58.47), 336.10 (M+H, 9.01). Anal. Calcd for $C_{17}H_{15}Cl_2NO_2$: C 60.73, H 4.50, N 4.17. Found: C 60.91, H 4.54, N 4.26.

4-(4-Chlorophenyl)-1-(4-methoxyphenyl)-3-(2-theinyl) azetidin-2-one ***(8e)*** Was prepared from *N*-(4-chlorobenzylidene)-4-methoxybenzenamine (**6c**) and acyl chloride **7**. Yield 54 %, buff crystals, mp 146–147 °C. IR (KBr, υ cm^{-1}): 3010 (CH aromatic), 2958, 2837 (CH aliphatic), 1739 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 3.69 (s, 3H, OCH_3), 4.70 (d, 1H, H-4, $J = 2.7$ Hz), 5.30 (d, 1H, H-3, $J = 2.7$ Hz), 6.89 (d, 2H, Ar–H, $J = 9$ Hz), 6.91–7.15 (m, 4H, Ar–H), 7.23 (d, 2H, Ar–H, $J = 9$ Hz), 7.48–7.55 (m, 3H, Ar–H); ^{13}C NMR (100 MHz, DMSO)δ ppm: 55.72 (C-3), 59.47 (OCH_3), 62.67 (C-4), 114.99 (2C), 119.03 (2C), 126.49, 126.72, 127.84, 128.90 (2C), 129.52 (2C), 130.57, 133.67, 136.55, 136.62, 156.36 (ArC), 164.02 (C=O); EIMS m/z (%rel. abundance): 368.26 (M−H, 12.52), 369.22 (M^+, 8.61); Anal. Calcd for $C_{20}H_{16}ClNO_2S$: C 64.95, H 4.36, N 3.79. Found: C 65.18, H 4.43, N 3.84.

3-Chloro-4-(furan-2-yl)-1-(4-methoxyphenyl)azetidin-2-one ***(8f)*** Was prepared from *N*-[(furan-2-yl)methylene]-4-methoxybenzenamine (**6e**) and acyl chloride **7**. Yield 64 %, brown oil. IR (KBr, υ cm^{-1}): 3001 (CH aromatic), 29254, 2927 (CH aliphatic), 1754 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 3.70 (s, 3H, OCH_3), 5.44 (s, 1H, H-4, $J = 2.1$ Hz), 5.47 (s, 1H, H-3, $J = 2.1$ Hz), 6.50–6.83 (m, 2H, furan H), 6.90 (d, 2H, Ar–H, $J = 9$ Hz), 7.26 (d, 2H, Ar–H, $J = 9$ Hz), 7.71 (d, 1H, furan H); ^{13}C NMR (100 MHz, DMSO)δ ppm: 55.75 (OCH_3), 58.17 (C-3), 60.34 (C-4), 111.48, 112.41, 115.01 (2C), 119.26 (2C), 130.06, 145.02, 147.70, 156.90 (ArC), 160.32 (C=O); EIMS m/z (%rel. abundance): 276.97 (M^+, 18.35), 278.98 (M+2, 6.59); Anal. Calcd for $C_{14}H_{12}ClNO_3$: C 60.55, H 4.36, N 5.04. Found: C 60.78, H 4.42, N 5.17.

4-(3,4,5-Trimethoxyphenyl)-1-(4-methoxyphenyl)-3-(2-thienyl)-azetidin-2-one ***(8g)*** Was prepared from *N*-(3,4,5-trimethoxybenzylidene)-4-methoxybenzenamine (**6d**) and

acyl chloride **7**. Yield 70 %, dark brown crystals, mp 130–131 °C. IR (KBr, υ cm^{-1}): 3003 (CH aromatic), 2927, 2839 (CH aliphatic), 1757 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm: 3.67 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.77 (s, 6H, $2OCH_3$), 4.83 (d, 1H, H-4, $J = 2$ Hz), 5.18 (d, 1H, H-3, $J = 2$ Hz), 6.87 (s, 2H, Ar–H), 6.90–7.54 (m, 7H, 4Ar–H and 3thienyl Hs); ^{13}C NMR (100 MHz, DMSO)δ ppm: 55.65 (C-3), 56.38 (OCH_3), 59.42 (2 OCH_3), 60.39 (OCH_3), 64.00 (C-4), 104.33 (2C), 114.91 (2C), 119.05 (2C), 126.09, 126.64, 127.83, 130.95, 133.11, 136.96, 138.03, 153.85 (2C), 156.31 (ArC), 164.43 (C=O); EIMS m/z (%rel. abundance): 425.03 (M^+, 11.38); Anal. Calcd for $C_{23}H_{23}NO_5S$: C 64.92, H 5.45, N 3.29. Found: C 65.08, H 5.53, N 3.41.

4-(2-Bromophenyl)-3-chloro-1-(3,4,5-trimethoxyphenyl)azetidin-2-one ***(8h)*** Was prepared from *N*-(2-bromobenzylidene)-3,4,5-trimethoxybenzenamine (**6f**) and acyl chloride **7.** Yield 42 %, grayish brown crystals, mp 118–119 °C. IR (KBr, υ cm^{-1}): 3016 (CH aromatic), 2995, 2981 (CH aliphatic), 1762 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm: 3.61 (s, 3H, OCH_3), 3.67 (s, 6H, $2OCH_3$), 5.31 (d, 1H, H-4, $J = 1.6$ Hz), 5.63 (d, 1H, H-3, $J = 1.6$ Hz), 6.57 (s, 2H, Ar–H), 7.32–7.43 (m, 3H, Ar–H), 7.76 (d, 1H, Ar–H, $J = 9$ Hz); ^{13}C NMR (100 MHz, DMSO)δ ppm: 56.32 (C-4), 60.57 (2 OCH_3), 62.28 (OCH_3) 64.51 (C-3), 96.02 (2C), 122.89, 128.82, 131.34 (2C), 132.67, 133.75, 134.32, 135.24, 153.79 (2C) (ArC), 160.86 (C=O); EIMS m/z (%rel. abundance): 424.84 (M^+, 15.83), 425.87 (M+H, 3.09), 426.85 (M+2, 20.42), 427.86 (M+3, 3.95), 428.84 (M+4, 5.52); Anal. Calcd for $C_{18}H_{17}BrClNO_4$: C 50.67, H 4.02, N 3.28. Found: C 50.79, H 4.08, N 3.41.

4-(2-Bromophenyl)-3-chloro-1-(3,4,5-trimethoxyphenyl)-3-methylazetidin-2-one ***(8i)*** Was prepared from *N*-(2-bromobenzylidene)-3,4,5-trimethoxybenzenamine (**6f**) and acyl chloride **7**. Yield 77 %, buff crystals, mp 150–151 °C. IR (KBr, υ cm^{-1}): 3001 (CH aromatic), 2927, 2889 (CH aliphatic), 1757 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 2.12 (s, 3H, CH_3), 3.63 (s, 3H, OCH_3), 3.67 (s, 6H, $2OCH_3$), 5.81 (s, 1H, H-4), 6.64 (s, 2H, Ar–H), 7.06–7.39 (m, 3H, Ar–H), 7.73–7.81 (d, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO)δ ppm: 24.88 (CH_3) 56.43 (2 OCH_3), 60.60 (OCH_3) 67.29 (C-3), 69.04 (C-4), 96.36 (2C), 123.12, 128.16, 128.50, 130.78, 131.04, 132.77, 133.77, 135.39, 153.76 (2C) (ArC), 163.85 (C=O); EIMS m/z (%rel. abundance): 438.95 (M^+, 1.48), 440.97 (M+2, 2.12); Anal. Calcd for $C_{19}H_{19}BrClNO_4$: C 51.78, H 4.35, N 3.18. Found: C 51.87, H 4.42, N 3.25.

4-(2-Bromophenyl)-1-(3,4,5-trimethoxyphenyl)-3-phenylazetidin-2-one ***(8j)*** Was prepared from *N*-(2-bromobenzylidene)-3,4,5-trimethoxybenzenamine (**6f**) and acyl chloride **7**. Yield 39 %, buff crystals, mp 126–127 °C. IR (KBr, υ cm^{-1}): 3001(CH aromatic), 2953, 2926 (CH aliphatic), 1743 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 3.60 (s, 3H, OCH_3), 3.65 (s, 6H, $2OCH_3$), 4.47 (d, 1H, H-4), 5.60 (d, 1H, H-3), 6.51 (s, 2H, Ar–H), 7.26–7.33 (m, 3H, Ar–H), 7.36–7.43 (m, 5H, Ar–H), 7.66 (d, 1H, Ar–H, $J = 7.8$ Hz); ^{13}C NMR (100 MHz, DMSO)δ ppm: 55.71 (C-3), 60.05 (C-4), 61.35 (2 OCH_3), 63.41 (OCH_3), 94.93 (2C), 122.316, 127.66 (2C), 127.74 (2C), 128.43 (2C), 128.77 (2C), 130.25, 132.84, 133.09, 134.79, 136.165, 153.28 (2C) (ArC), 165.008 (C=O); EIMS m/z (%rel. abundance): 466.86 (M^+, 4.06), 468.97 (M+2, 5.07); Anal. Calcd for $C_{24}H_{22}BrNO_4$: C 61.55, H 4.73, N 2.99. Found: C 61.70, H 4.82, N 3.08.

4-(2-Bromophenyl)-1-(3,4,5-trimethoxyphenyl)-3-(2-thienyl)azetidin-2-one ***(8k)*** Was prepared from *N*-(2-bromobenzylidene)-3,4,5-trimethoxybenzenamine (**6f**) and acyl chloride **7**. Yield 61 %, buff crystals, mp 109–110 °C. IR (KBr, υ cm^{-1}): 3066 (CH aromatic), 2951, 2825 (CH aliphatic), 1764 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm: 3.60 (s, 3H, OCH_3), 3.66 (s, 6H, 2 OCH_3), 4.83 (d, 1H, H-4, $J = 2.4$ Hz), 5.58 (d, 1H, H-3, $J = 2.4$ Hz), 6.56 (s, 2H, Ar–H), 7.07–7.56 (m, 6H, 3Ar–H and 3H thienyl H), 7.72 (d, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO)δ ppm: 56.25 (C-3), 58.96 (C-4), 60.58 (2 OCH_3), 62.96 (OCH_3), 95.43 (2C), 122.93, 126.49, 127.15, 127.79, 129.02, 130.99 (2C), 133.24, 133.66, 134.76, 136.01, 136.68, 153.81 (2C) (ArC), 164.50 (C=O); EIMS m/z (%rel. abundance): 472.82 (M^+, 33.19), 474.83 (M+2, 34.90); Anal. Calcd for $C_{22}H_{20}BrNO_4S$: C 55.70, H 4.25, N 2.95. Found: C 55.84, H 4.31, N 3.03.

4-(3-Bromophenyl)-1-(3,4,5-trimethoxyphenyl)-3-(2-thienyl)azetidin-2-one ***(8l)*** Was prepared from *N*-(3-bromobenzylidene)-3,4,5-trimethoxybenzenamine (**6g**) and acyl chloride **7**. Yield 30 % (65 % by microwave method), buff crystals, mp 118–119 °C. IR (KBr, υ cm^{-1}): 3001 (CH aromatic), 2958, 2924 (CH aliphatic), 1751; (C=O) ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm: 3.59 (s, 3H, OCH_3), 3.66 (s, 6H, $2OCH_3$), 4.87(d, 1H, H-3, $J = 2.3$ Hz), 5.36 (d, 1H, H-4, $J = 2.3$ Hz), 6.61 (s, 2H, Ar–H), 7.06-7.08 (m, 1H, thienyl-H), 7.16 (d, 1H, thienyl-H), 7.36–7.40 (t, 1H, thienyl-H, $J = 7.84$ Hz), 7.53–7.58 (m, 3H, Ar–H), 7.80 (s, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO)δ ppm: 56.23 (C-3), 58.96 (2 OCH_3), 60.57 (OCH_3), 62.78 (C-4) 95.62 (2C), 122.72, 126.29, 126.56, 126.84, 127.86, 130.09, 131.67, 132.13, 133.18, 134.68, 136.41, 140.21, 153.71 (2C) (ArC), 164.53 (C=O); EIMS m/z (%rel. abundance): 472.90 (M^+, 7.63), 474.91 (M+2, 8.90); Anal. Calcd for $C_{22}H_{20}BrNO_4S$: C 55.70, H 4.25, N 2.95. Found: C 55.89, H 4.31, N 3.07.

4-(4-Bromophenyl)-1-(3,4,5-trimethoxyphenyl)-3-phenylazetidin-2-one ***(8m)*** Was prepared from *N*-(4-bromobenzylidene)-3,4,5-trimethoxybenzenamine (**6h**) and acyl chloride **7**. Yield 19 % (45 % by microwave method), yellow crystals, mp 113–114 °C. IR (KBr, υ cm^{-1}): 3001 (CH aromatic), 2954, 2850 (CH aliphatic), 1741 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 3.59 (s, 3H, OCH_3), 3.65 (s, 6H, $2OCH_3$), 4.47(d, 1H, H-4), 5.34 (d, 1H, H-3), 6.61 (s, 2H, Ar–H), 7.38–7.41 (m, 5H, Ar–H), 7.47 (d, 2H, Ar–H, $J = 7.8$ Hz), 7.60 (d, 2H, Ar–H, $J = 7.8$ Hz); ^{13}C NMR (100 MHz, DMSO)δ ppm: 55.75 (C-3), 60.04 ($2OCH_3$), 61.32 (OCH_3), 63.43 (C-4), 95.16 (2C), 121.64, 127.50, 127.73 (2C), 128.83 (2C), 128.87 (2C), 131.89 (2C), 132.78, 134.12, 134.60, 136.99, 153.18 (2C) (ArC), 165.015 (C=O); EIMS m/z (%rel. abundance): 468.15 (M^+, 73.91), 469.15 (M+H, 13.59). Anal. Calcd for $C_{24}H_{22}BrNO_4$: C 61.55, H 4.73, N 2.99. Found: C 61.72, H 4.80, N 3.03.

4-(4-Bromophenyl)-1-(3,4,5-trimethoxyphenyl)-3-(2-thienyl)azetidin-2-one ***(8n)*** Was prepared from *N*-(4-bromobenzylidene)-3,4,5-trimethoxybenzenamine (**6h**) and acyl chloride **7**. Yield 39 % (56 % by microwave method), pale brown crystals, mp 173–174 °C. IR (KBr, υ cm^{-1}): 3111 (CH aromatic), 2954, 2823 (CH aliphatic), 1759 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 3.58 (s, 3H, OCH_3), 3.65 (s, 6H, $2OCH_3$), 4.77 (d, 1H, H-4, $J = 2.4$ Hz), 5.34 (d, 1H, H-3, $J = 2.4$ Hz), 6.59 (s, 2H, Ar–H), 7.06–7.15 (m, 3H, thienyl Hs), 7.48 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.61 (d, 2H, Ar–H, $J = 8.4$ Hz); ^{13}C NMR (100 MHz, DMSO)δ ppm: 56.26 (C-3), 59.08 (2 OCH_3), 60.56 (OCH_3), 62.90 (C-4), 95.65 (2C), 122.35, 126.55, 126.79, 127.85, 129.37 (2C), 132.42 (2C), 133.20, 134.67, 136.52, 136.93, 153.71 (2C) (ArC), 164.51 (C=O); EIMS m/z (%rel. abundance): 472.88 (M^+, 26.64), 474.88 (M+2, 27.97); Anal. Calcd for $C_{22}H_{20}BrNO_4S$: C 55.70, H 4.25, N 2.95. Found: C 55.89, H 4.28, N 3.01.

4-(4-Chlorophenyl)-1-(3,4,5-trimethoxyphenyl)-3-phenylazetidin-2-one ***(8o)*** Was prepared from *N*-(4-chlorobenzylidene)-3,4,5-trimethoxybenzenamine (**6i**) and acyl chloride **7**. Yield 22 % (49 % by microwave method), yellow crystals, mp 97–98 °C. IR (KBr, υ cm^{-1}): 3030 (CH aromatic), 2997, 2933(CH aliphatic), 1743 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 3.59 (s, 3H, OCH_3), 3.65 (s, 6H, $2OCH_3$), 4.48 (d, 1H, H-4, $J = 2.1$ Hz), 5.36 (d, 1H, H-3, $J = 2.1$ Hz), 6.61 (s, 2H, Ar–H), 7.36–7.42 (m, 5H, Ar–H), 7.47 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.55 (d, 2H, Ar–H, $J = 8.4$ Hz); ^{13}C NMR (100 MHz, DMSO)δ ppm: 56.25 (C-3), 60.56 (2 OCH_3), 61.81 (OCH_3), 63.90 (C-4), 95.60 (2C), 122.16, 128.03 (2C), 129.38 (2C), 129.40 (4C), 132.41, 133.28, 134.55, 135.12, 137.49, 153.68 (2C) (ArC), 165.56 (C=O); EIMS m/z (%rel. abundance): 423.12 (M^+, 0.89), 425.14 (M+2, 0.86); Anal. Calcd for $C_{24}H_{22}ClNO_4$: C 68.00, H 5.23, N 3.30. Found: C 68.14, H 5.31, N 3.43.

4-(4-Chlorophenyl)-1-(3,4,5-trimethoxyphenyl)-3-(2-thienyl)azetidin-2-one ***(8p)*** Was prepared from *N*-(4-chlorobenzylidene)-3,4,5-trimethoxybenzenamine (**6i**) and acyl chloride **7**. Yield 89 %, buff crystals, mp 152–153 °C. IR (KBr, υ cm^{-1}): 3111 (CH aromatic), 2954, 2825 (CH aliphatic), 1761 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 3.58 (s, 3H, OCH_3), 3.65 (s, 6H, $2OCH_3$), 4.78 (d, 1H, H-4, $J = 2.4$ Hz), 5.35 (d, 1H, H-3, $J = 2.4$ Hz), 6.58 (s, 2H, Ar–H), 7.06–7.15 (m, 3H, thienyl), 7.47 (d, 2H, Ar-H, $J = 8.4$ Hz), 7.55 (d, 2H, Ar-H, $J = 8.4$ Hz); ^{13}C NMR (100 MHz, DMSO)δ ppm: 56.25 (C-3), 59.12 ($2OCH_3$), 60.55 (OCH_3), 62.85 (C-4), 95.65 (2C), 126.54, 126.78, 127.85, 129.09 (2C), 129.50 (2C), 133.21, 133.77, 134.66, 136.50, 136.53, 153.70 (2C) (ArC), 164.51 (C=O); EIMS m/z (%rel. abundance): 429.15 (M^+, 17.50), 431.10 (M+2, 6.79). Anal. Calcd for $C_{22}H_{20}ClNO_4S$: C 61.46, H 4.69, N 3.26. Found: C 61.62, H 4.73, N 3.42.

Biological study

Measurement of cytotoxicity by SRB assay

The cytotoxic activity of the newly synthesized compounds was measured in vitro using SRB assay according to the previously reported method (Skehan et al. 1990). Cells were plated in 96-multiwell microtiter plates (10^4 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cells to the walls of the plates. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the test compounds (0, 5, 12, 25, and 50 μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37 °C in an atmosphere of 5 % CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4 % (wt/vol) with SRB dissolved in 1 % acetic acid. Unbound dye was removed by four washes with 1 % acetic acid, and attached stain was recovered with 2 Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relationship between surviving fraction and drug concentration was plotted to get the survival curve. The concentration required for 50 % inhibition of cell viability (IC_{50}) was calculated. The results are given in Tables 1 and 3 and graphically presented in Fig. 1.

In vitro tubulin polymerization assay

MCF-7 and HCT-116 cells were obtained from American Type Culture Collection, and cultured using DMEM

Table 1 In vitro cytotoxic activitiy of the tested compounds **8a–p** on MCF-7 and HCT-116 cell lines

Compound no.	MCF-7 (IC_{50})[a] (μM)	HCT (IC_{50})[a] (μM)
8a	11.18	11.67
8b	21.80	22.67
8c	9.27	28.09
8d	20.82	41.64
8e	10.81	16.84
8f	36.01	22.97
8g	8.22	17.46
8h	9.37	8.62
8i	18.15	10.39
8j	7.25	6.25
8k	28.45	8.70
8l	156.2	185.7
8m	8.54	20.02
8n	6.74	7.44
8o	7.07	8.68
8p	10.93	9.25
Colchicine	12.35	3.8

[a] IC_{50} dose of the compound that inhibits tumor cell proliferation by 50 %

(Invitrogen/Life Technologies) supplemented with 10 % FBS (Hyclone), 10 ug/mL of insulin (Sigma-Aldrich, MO, USA), and 1 % penicillin–streptomycin. All of the other chemicals and reagents were purchased from Sigma, or Invitrogen. Plate cells (cells density 1.2–1.8 × 10,000 cells/well) in a volume of 100 μL complete growth medium +100 ul of the tested compound with the IC_{50} conc. per well in a 96-well plate for 24 h before the enzyme assay for tubulin.

Tubulin polymerization inhibitory activity was determined using human type β-tubulin SEB870HU assay kit (Cloud-Clone Corp. USA). The procedure of the used kit was performed according to the manufacturer's instructions.

Molecular modeling study

The PDB (code: 1SA0) was chosen as the template for the modeling study of compounds **8n** and **8o** bound to colchicines-binding site. The crystal structure was obtained from RCSB protein data bank (http://www.rcsb.org/pdb/home/home.do). The molecular docking study was carried out using CDOCKER protocol within Discovery Studio version 2.55. For protein preparation, only chain A and B with co crystallized Colchicine **1** was retained, the hydrogen atoms were added and optimized, the water molecules and impurities were removed. For ligand preparation, the 3D structures of the tested compounds were built using ChemBioDraw Ultra 11.0 and minimized using discovery studio 2.55. The validation results showed the same binding interactions of the co-crystallized and the re-docked ligand with rmsd of 0.95 Å The docking study was performed by inserting the tested compounds into the colchicines-binding site. The type of interactions between the compounds and protein were analyzed.

Results and discussion

Chemistry

The preparation of target combretastatin-based analogues **8a–p** was based upon incorporation of 4-methoxyphenyl or 3,4,5-trimethoxyphenyl scaffold at *N*-1 position of the β-lactam ring (mimicking ring A of CA-4) and a substituted phenyl ring at C-4 of the β-lactam ring (mimicking ring B of CA-4), (Fig. 1). As shown in Scheme 1, the synthesis firstly involves the formation of the imine precursors **6a–i** by condensation of the appropriately substituted primary aromatic amines **4** and certain substituted benzaldehydes **5**

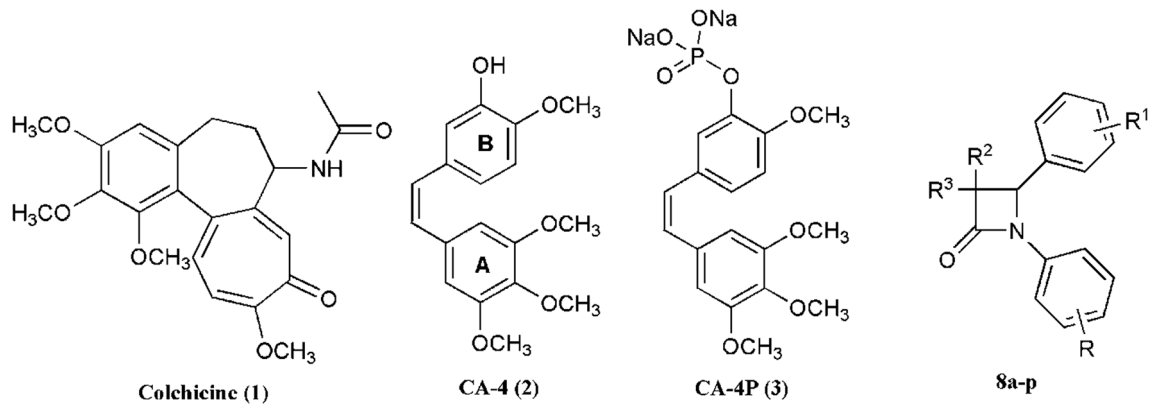
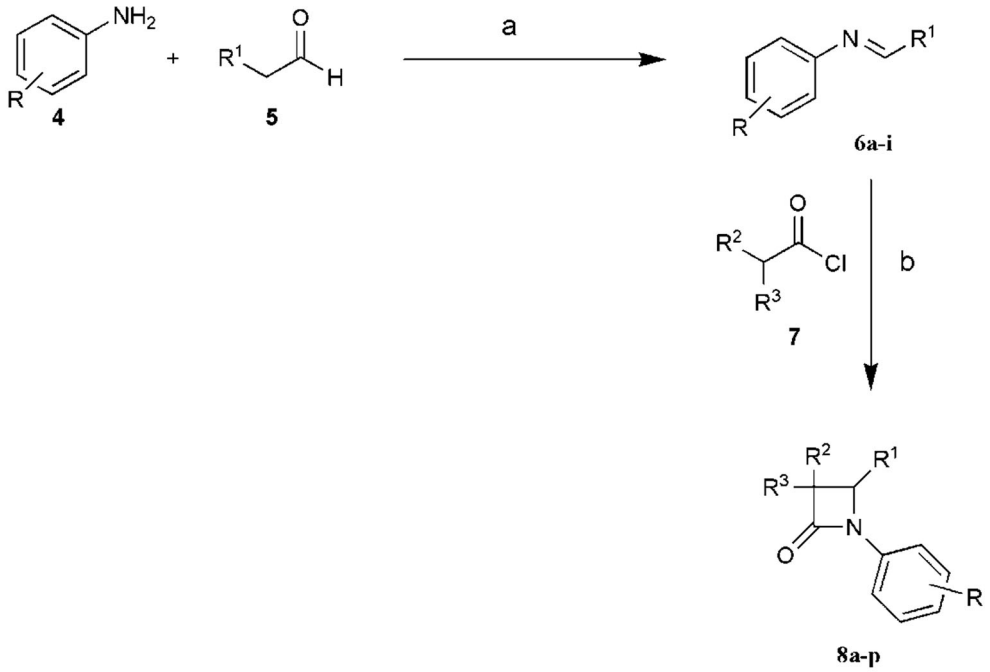


Fig. 1 Some tubulin binding agents and the newly synthesized compounds **8a–p**

to give the desired imines in high decent yields. The target β-lactam derivatives **8a–p** were obtained adopting the Staudinger cycloaddition reaction, which initiated by the nucleophilic attack of the imine derivatives **6a–i** to an insitu generated ketene (from an appropriate substituted acetyl chloride **7** and TEA) following previously reported procedure (Banik et al. 2005) (Scheme 1). Stereochemistry of **8a–p** depends upon several factors; reaction conditions, order of addition of the reagents and substituents present on both the acid chloride and the imine (Xu 2009). In this work, the target compounds **8a–p** showed *trans* arrangement for H-3 and H-4 as evidenced by a pair of coupled doublets with coupling constants about, $J = 2$ Hz. No *cis* isomers were isolated in this series, likely due to steric hindrance between the substituents in 3- and 4-positions of the azetidin-2-one ring. Some β-lactam derivatives, namely **8l–o**, were synthesized adopting microwave irradiation by applying Microwave-induced Organic Reaction Enhancement (MORE) chemistry technique, starting with the appropriate imine precursors **6g–i** and certain substituted acetyl chlorides **7** in chlorobenzene and TEA for 10 min (Bose et al. 1995; Bandyopadhyay and Banik 2010) (Scheme 2). The β-lactam derivatives prepared by microwave irradiation technique were isolated in high yields with stereochemistry similar to that obtained by traditional methods.



6	R	R¹
a	4-OCH_3	2-BrC_6H_4
b	4-OCH_3	4-BrC_6H_4
c	4-OCH_3	4-ClC_6H_4
d	4-OCH_3	3,4,5 $(OCH_3)_3C_6H_2$
e	4-OCH_3	2-furyl
f	3,4,5 $(OCH_3)_3$	2-BrC_6H_4
g	3,4,5 $(OCH_3)_3$	3-BrC_6H_4
h	3,4,5 $(OCH_3)_3$	4-BrC_6H_4
i	3,4,5 $(OCH_3)_3$	4-ClC_6H_4

8	R	R¹	R²	R³
a	4-OCH_3	2-BrC_6H_4	Cl	H
b	4-OCH_3	2-BrC_6H_4	CH_3	Cl
c	4-OCH_3	4-BrC_6H_4	Cl	H
d	4-OCH_3	4-ClC_6H_4	CH_3	Cl
e	4-OCH_3	4-ClC_6H_4	2-thienyl	H
f	4-OCH_3	2-furyl	Cl	H
g	4-OCH_3	3,4,5- $(OCH_3)_3C_6H_2$	2-thienyl	H
h	3,4,5- $(OCH_3)_3$	2-BrC_6H_4	Cl	H
i	3,4,5- $(OCH_3)_3$	2-BrC_6H_4	CH_3	Cl
j	3,4,5- $(OCH_3)_3$	2-BrC_6H_4	phenyl	H
k	3,4,5- $(OCH_3)_3$	2-BrC_6H_4	2-thienyl	H
l	3,4,5- $(OCH_3)_3$	3-BrC_6H_4	2-thienyl	H
m	3,4,5- $(OCH_3)_3$	4-BrC_6H_4	phenyl	H
n	3,4,5- $(OCH_3)_3$	4-BrC_6H_4	2-thienyl	H
o	3,4,5- $(OCH_3)_3$	4-ClC_6H_4	phenyl	H
p	3,4,5- $(OCH_3)_3$	4-ClC_6H_4	2-thienyl	H

Scheme 1 Reagents and conditions: **a** ethanol, reflux, 5–12 h; **b** CH_2Cl_2, TEA, reflux, 7–12 h

Biological result

Cytotoxic activity

The newly synthesized β-lactam derivatives **8a–p** were screened for their cytotoxic activity against human breast cancer MCF-7 and the human colon cancer HCT-116 cell lines, using sulphorhodamine B (SRB) stain colorimetric assay. The drug concentration required to inhibit the cell growth by 50 % (IC_{50}) was determined and the results were displayed and represented graphically (Table 1; Fig. 2).

For comparison, the IC_{50} of the standard compound, colchicine, was evaluated. The results revealed that all tested compounds displayed cytotoxic activity against both tested cell lines. Compounds **8a**, **8c**, **8e**, **8g**, **8h**, **8j**, **8m**, **8n**, **8o** and **8p** showed cytotoxic activity against MCF-7 cell line superior to colchicine with IC_{50} range 6.74-11.18 μM, while compounds **8b**, **8d**, **8i** and **8k** demonstrated moderate activity. On the other hand, weak cytotoxic effect was observed by the compounds **8f** and **8l**. Further, the β-lactam derivatives **8a**, **8h**, **8i**, **8j**, **8k**, **8n**, **8o** and **8p** were found to be the most potent against HCT-116 cell line with IC_{50} range 6.25–11.67 μM.

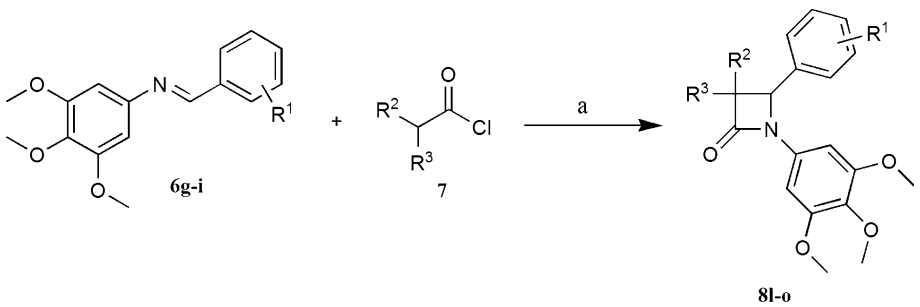
Based on the cytotoxicity data, the structure–activity relationship (SAR) for these trisubstituted azetidin-2-one derivatives has been elucidated. Keeping constant the 3,4,5-trimethoxyphenyl ring at *N*-1 and 2-bromophenyl substituent at C-4, the effect of modifications on the position 3 of the azetidin-2-one ring has been examined in compounds **8h**, **8i**, **8j** and **8k**. Compound **8j** having a phenyl substituent exhibited very good anticancer efficacy towards both MCF-7 and HCT-116 cell lines (IC_{50} = 7.25 and 6.25 μM) respectively. Slight reduction in cytotoxic activity was observed when the phenyl group was replaced by chloro substituent (**8h**). Further decrease in the activity against MCF-7 and HCT-116 cell lines was observed in the compound **8i** containing both chloro and methyl groups at C-3 of the ring. Compound **8k** with 2-thienyl substituent at C-3 showed the weakest activity among this series of compounds against MCF-7, but relatively good activity against HCT-116 cell line (IC_{50} = 8.70 μM). Among the series of compounds **8k**, **8l**, **8n**, **8p**, with 3,4,5-trimethoxyphenyl at *N*-1 and 2-thienyl substituent at C-3, it was obvious that the position of the substituents on the phenyl ring at C-4 of the azetidin-2-one ring is critical on the cytotoxicity of these compounds. It was observed that compounds with bromo or chloro substituents at *ortho* or *para* position of the phenyl ring displayed excellent to moderate cytotoxic activity with IC_{50} range of 6.74–28.45 μM against both MCF-7 and HCT-116 cell lines. On the other hand, compound **8l** with bromo substituent at *meta* position showed dramatic reduction in cytotoxicity against both tested human cancer cell lines.

In conclusion, the most active azetidin-2-one derivatives contain at C-4 of the ring either 3,4,5-trimethoxyphenyl substitution or 2- or 4-bromo or chloro substituted phenyl ring. Moreover, compounds with 3,4,5-trimethoxy phenyl substituents at *N*-1 showed generally better cytotoxic activity compared with the compounds having 4-methoxyphenyl substituent. On the other hand, compounds which are disubstituted at C-3 were found to display weaker cytotoxic activity compared with their monosubstituted analogues.

Tubulin polymerization inhibition assay

The most potent compounds in SRB assay, **8g**, **8j**, **8n** and **8o**, were further investigated for inhibition of tubulin polymerization aganist MCF-7 and HCT-116 cancer cell lines by applying enzyme linked immunosorbent assay (ELISA) using human *β*-tubulin assay kit SEB870Hu obtained from Cloud-Clone Corp. USA. The inhibitory

Scheme2 Reagents and conditions: **a** chlorobenzene, TEA, microwave, 10 min



8	R^1	R^2	R^3
l	3-Br	2-thienyl	H
m	4-Br	phenyl	H
n	4-Br	2-thienyl	H
o	4-Cl	phenyl	H

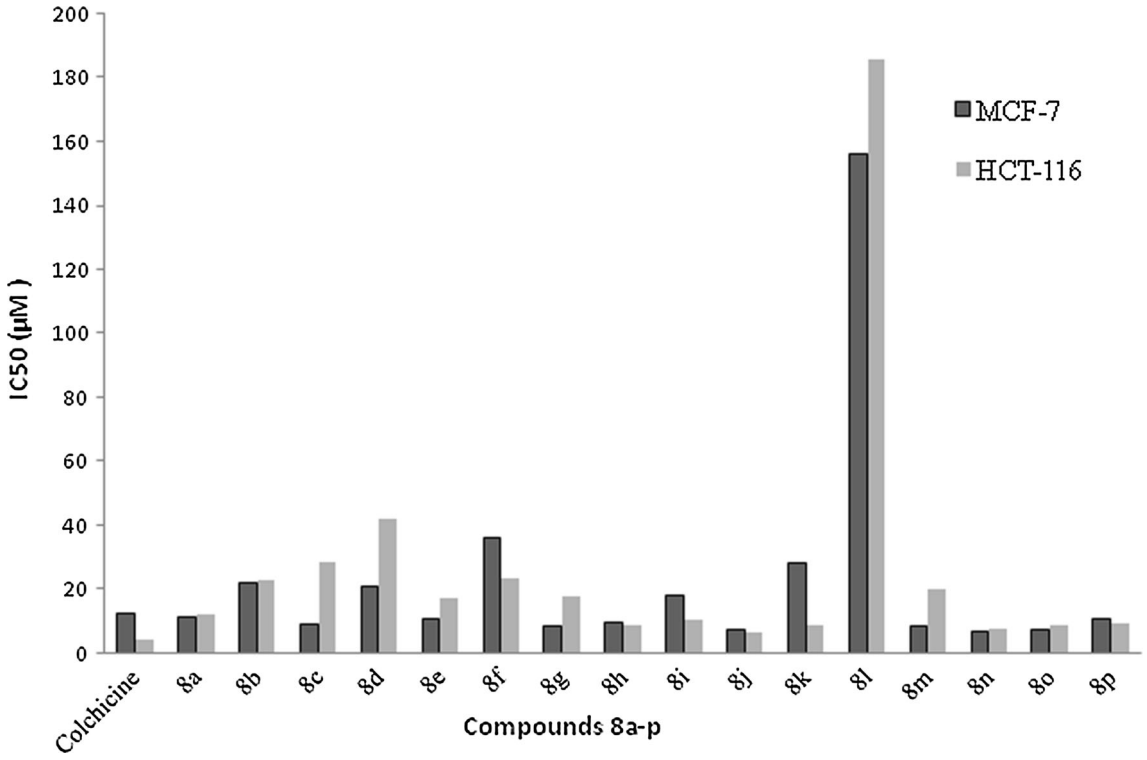


Fig. 2 Cytotoxic activity of the tested compounds **8a–p** on MCF-7 and HCT-116 cell lines

Table 2 Percentage inhibition of tubulin polymerization on human MCF-7 and HCT-116 cell lines at IC_{50} for the compounds **8g**, **8j**, **8n** and **8o**

Compound no.	IC_{50} (μM) MCF-7	% inhibition of tubulin polymerization (MCF-7)	IC_{50} (μM) HCT-116	% inhibition of tubulin polymerization (HCT-116)
8g	8.22	85.18	17.46	83.63
8j	7.25	81.12	6.25	85.60
8n	6.74	79.10	7.44	86.86
8o	7.07	78.11	8.68	84.83
Colchicine	12.35	86.90	3.8	84.41

activity of tubulin was given as the percentage inhibition at IC_{50} concentration of each compound (Table 2). The results showed that all tested compounds produced significant tubulin suppression compared to the reference antimitotic agent colchicine. Therefore, cytotoxic activity of the tested compounds may be referred to their anti-tubulin activity.

Evaluation of toxicity in normal cells

Equally important, compounds **8g**, **8j**, **8n** and **8o** were further investigated for toxicity on normal breast Hs371.T cell line and normal colon CCD18co cell line using sulphorhodamine B (SRB) stain colorimetric assay. Results in Table 3 revealed that compounds **8g** and **8o** displayed the lowest toxicity on normal Hs371.T in comparison to colchicines as the reference compound.

Molecular modeling studies

Molecular modeling simulation study was performed to investigate the probable binding modes of the most active

Table 3 *In vitro* cytotoxic activity of the tested compounds **8g**, **8j**, **8n** and **8o** on Hs371.T and CCD18co cell lines

Compound no.	IC_{50} (μM)[a]	
	Hs371.T	CCD18co
8g	88.8	38.13
8j	43.23	15.88
8n	44.03	30.44
8o	77.38	29.57
Colchicine	67.21	58.12

[a] IC_{50} dose of the compound that inhibits tumor cell proliferation by 50 %

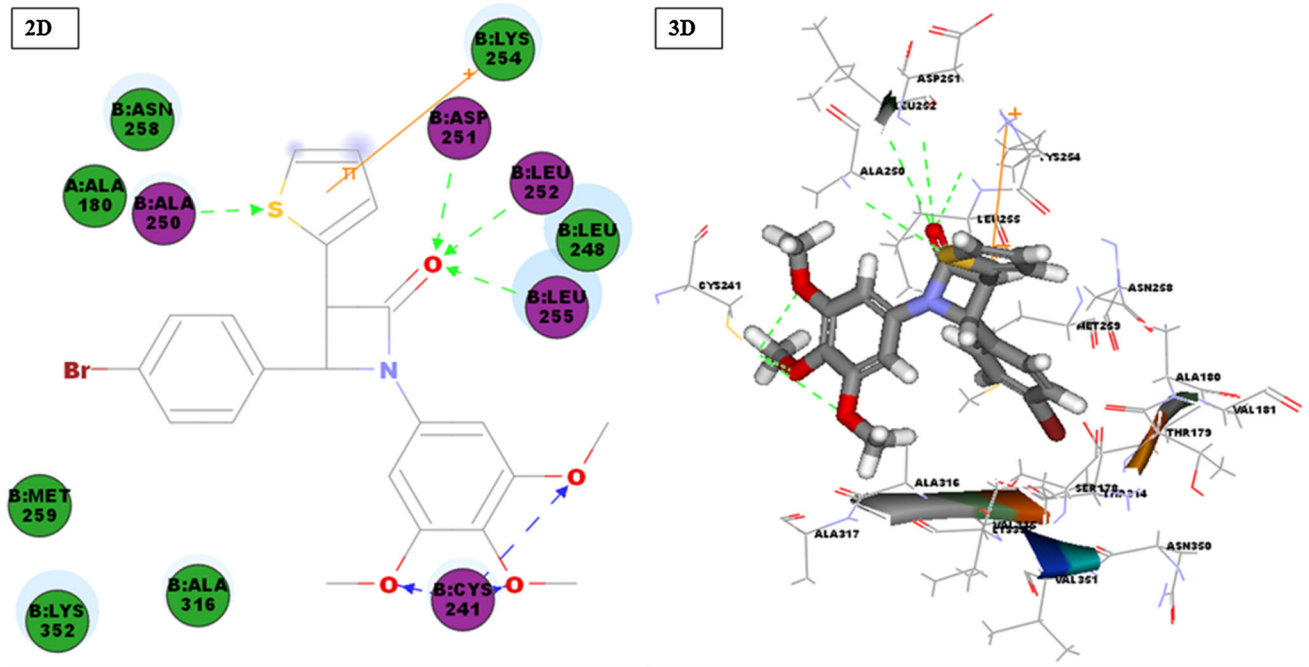


Fig. 3 2D and 3D interaction of compound **8n** in the colchicine binding site of tubulin (PDB code 1SA0); showing key hydrogen bonding interactions (*dotted lines*)

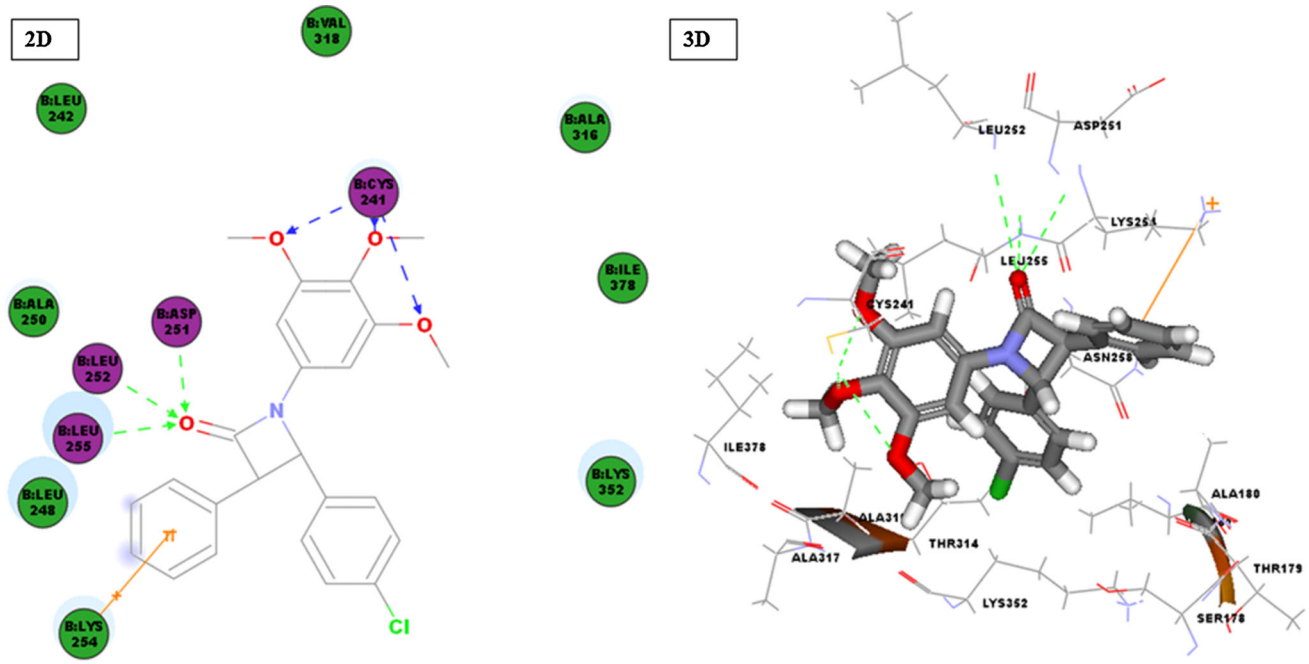


Fig. 4 2D and 3D interaction of compound **8o** in the colchicine binding site of tubulin (PDB code 1SA0); showing key hydrogen bonding interactions (*dotted lines*)

compounds, **8n** and **8o**, using the reported X-ray structure of tubulin co-crystallized with *N*-deacetyl-*N*-(2-mercapto acetyl) colchicine (DAMA colchicine, PDB code: 1SA0) (Wei et al. 2004). The colchicine-binding site was mainly buried in the β-subunit of tubulin, in addition to some limited interaction with the α-subunit, while surrounded by H7 and H8 α-helices, S8 and S9 β-strands and T7 loop, which interact with the colchicine-site ligand (Dorleans et al. 2009). Researches have proved that the most important residues for colchicine-binding are Val318 and

Cys241. Methoxy group in ring A of colchicine can be cross linked with Cys241 (Andreu et al. 1998). Also Thr179, Lys 352, Val 181, Leu248 and Ala250 have been reported to play a crucial role in the interaction of azetidin-2-ones with the colchicines-binding site (O'Boyle et al. 2011b). Results obtained from docking imitation were presented in Figs. 3 and 4. Hydrogen bonding interaction between Cys 241 and methoxy group of the compounds **8n** and **8o** was found to be comparable to colchicine. These binding parallels may rationalize the cytotoxic potency observed by these compounds. Also, hydrogen bonding interaction between oxygen at C-2 and Asp 251, Leu 252, Leu 255 may rationalize the increased activity of these compounds. In addition, compound **8n** showed hydrogen bonding interaction between thiophene ring sulfur and Ala 250, (Fig. 3). Moreover, strong π–π bond stabilizes the interaction between Lys 254 amino acid residue and the aromatic ring system at C-3 in these compounds.

Conclusion

In this research, new series of azetidin-2-one derivatives **8a**–**p** were synthesized with the aim to rigidify the traditionally isomerizable double bond in CA-4 that suppresses its activity. The synthesized compounds were evaluated for their cytotoxic activity against MCF-7 and HCT-116 cancer cell lines. It was obvious that, presence of 3,4,5-trimethoxy phenyl scaffold at *N*1 or C-4 and a 2-thienyl or phenyl group at C-3 was found to increase the cytotoxic activity of these compounds. Four compounds **8g**, **8j**, **8n** and **8o** showed higher percentage suppression of tubulin (79.09, 78.11, 81.12 and 85.18) respectively in comparison with colchicine against MCF-7 cell line and (86.86, 86.83, 85.63 and 83.63) respectively against HCT-116 cell line. Molecular modeling studies were performed to rationalize the observed cytotoxic activity by the synthesized compounds. These compounds could be considered a promising leads for further investigation.

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