Discovery of novel quinoline-based analogues of combretastatin A-4 as tubulin polymerisation inhibitors with apoptosis inducing activity and potent anticancer effect

Tarek S. Ibrahim, Mohamed M. Hawwas, Azizah M. Malebari, Ehab S. Taher, Abdelsattar M. Omar, Thikryat Neamatallah, Zakaria K. Abdel-Samii, Martin K. Safo, and Yaseen A. M. M. Elshaier

ABSTRACT

A new series of quinoline derivatives of combretastatin A-4 have been designed, synthesised and demonstrated as tubulin polymerisation inhibitors. These novel compounds showed significant antiproliferative activities, among them, 12c exhibited the most potent inhibitory activity against different cancer cell lines (MCF-7, HL-60, HCT-116 and HeLa) with IC50 ranging from 0.010 to 0.042 nM, and with selectivity profile against MCF-10A non-cancer cells. Further mechanistic studies suggest that 12c can inhibit tubulin polymerisation and cell migration, leading to G2/M phase arrest. Besides, 12c induces apoptosis via a mitochondrial-dependant apoptosis pathway and caused reactive oxygen stress generation in MCF-7 cells. These results provide guidance for further rational development of potent tubulin polymerisation inhibitors for the treatment of cancer.

HIGHLIGHTS

• A novel series of quinoline derivatives of combretastatin A-4 have been designed and synthesised.
• Compound 12c showed significant antiproliferative activities against different cancer cell lines.
• Compound 12c effectively inhibited tubulin polymerisation and competed with [3H] colchicine in binding to tubulin.
• Compound 12c arrested the cell cycle at G2/M phase, effectively inducing apoptosis and inhibition of cell migration.

GRAPHICAL ABSTRACT

isosteric replacement of ring B with quinoline

CA-4

12c

IC50 10-42 nM against different cancer cell lines
1. Introduction

Cancer is a disease of an uncontrolled growth and abnormal division of cells, which leads to death. In recent years, targeted antineoplastic agents have become an effective treatment choice for cancer, with pharmaceutical companies focusing on targeted therapies against different and special cancer types. Tubulin polymerisation inhibitors represent one of the most well-known and potential examples of such targeted cancer therapies. Tubulin is a globular protein that performs a substantial function in cell mitosis. Microtubules (MTs), which represent the basic constituents of eukaryotic cell, are cytoskeletons constructed by the association of α- and β-tubulin heterodimers with a head and tail pattern to form hollow cylindrical tubes (nearly 25 nm in diameter). MTs play a crucial role in many fundamental cellular activities, such as motility, cell formation, cell secretion, signalling, maintenance of cell shape, regulation of intracellular transport and cell division. Due to these multiple functions, microtubule system has become an attractive target for cancer chemotherapy. Disruption of MTs or tubulin dynamics exposes the cell to mitotic arrest of the cell cycle at G2/M phase, and consequently induction of cellular apoptosis. Several microtubule-interfering agents (MIAs) have been identified, e.g. paclitaxel, vincristine, and colchicine that are obtained from the natural products, taxol, vinca and colchicine, respectively. MIAs are known to bind to tubulin at specific binding sites that are classified as taxol, vinca and colchicine sites to either enhance or inhibit tubulin polymerisation. For example, microtubule stabilisers, e.g. paclitaxel stimulate microtubule polymerisation, while microtubule destabilizers, e.g. colchicine, and the vinca alkaloids vinblastine and vincristine inhibit polymerisation of microtubules.

Significant attention is now focused on colchicine binding site inhibitors due to their positive impact on ABC-transporter-mediated drug resistance. Combretastatin A-4 (CA-4), (1, Figure 1) has been reported as the most potent antimitotic agent of this family against several tumour cells. CA-4 was first isolated from the bark of the willow tree Combretum caffrum from South Africa in 1989. CA-4 has a vascular disrupting activity against tumour cell vasculature by preventing blood supply to solid tumour, resulting in apoptosis. Given its structural simplicity, CA-4 has been studied as a lead pharmacophore for deciphering tubulin functions and properties. Phases II and III clinical studies are currently ongoing with tubulin-targeted drugs. Structure activity relationships (SAR) studies with CA-4 have revealed three important structural features (Figure 1). These include: (i) a 3,4,5-trimethoxy moiety on ring A that is essential for activity; (ii) a cis-configuration of both aromatic rings that is essential for activity (trans-orientation is inactive); (iii) the presence of a small substituent on ring B, e.g. methoxy group that is important for activity. The cis-alkene configuration in CA-4 allows the aromatic rings to assume optimal binding orientation for interactions with the colchicine binding site. Unfortunately, the cis configuration of CA-4 has a propensity for undergoing transformation to the inactive trans configuration upon storage and during in vivo metabolism.

To overcome this, many structural modifications of CA-4 have been undertaken where the cis double bond is replaced with heterocycles, either monocyclic, such as oxadiazole, isoxazole and imidazole, resulting in compounds, such as 1, 2 and 3 respectively (Figure 1) or fused heterocyclic, such as pyrazolopyridines, triazolopyridines and triazolothiadiazine derivatives. These compounds, like CA-4 showed pronounced activity against a panel of cancer cell lines.

Quinoline derivatives are popular for the treatment of malaria. Moreover, quinoline heterocyclic containing compounds demonstrate potent anticancer activities with different modes of actions, including inhibition of proteasome, tyrosine kinases, and...
Previous studies have reported the antiproliferative activity of CA-4, isoCA-4 or chalcone compounds containing quinoline scaffold, either as ring A bioisoster, e.g. 4a and 4b or ring B bioisoster, e.g. 4c – 4d. These compounds demonstrate the potential of the quinoline ring as a template for developing more promising tubulin polymerisation inhibitors and antiproliferative agents.

In this work, we optimised CA-4 into a series of novel hybrid quinoline derivatives as potent tubulin inhibitor, which involves introducing a rigid oxazolone or imidazolone between rings A and B to maintain the cis configuration, as well as targeting the quinolyl moiety (ring B), by varying the electronic substituents effect while maintaining the 3,4,5-trimethoxyphenyl moiety as present in ring A of CA-4 (Figure 2). Following, we synthesised several analogues that constitute two classes of compounds: the oxazolones (Compounds 12a–h) and the imidazolones (Compounds 13a–h). The compounds have been screened for their antiproliferative activities against a variety of cancer cell lines, as well as studied for their mechanism of action. We expect the results to lead to better understanding of the mechanistic mode of the compounds’ activity against tubulin and provide guidance for further development of potent anticancer drugs.

2. Experimental section

2.1. Chemistry

Melting points were determined with a Gallenkamp (London, UK) melting point apparatus and are uncorrected. IR spectra (KBr, cm⁻¹) were recorded on Bruker Vector, 22FT-IR [Fourier Transform Infra-red (FTIR), Germany] spectrometer. Unless otherwise specified, proton (1H) and carbon (13C) NMR spectra were recorded at room temperature in base filtered CDCl₃ on a spectrometer operating at 400 & 300 MHz for proton and 100 & 75 MHz for carbon nuclei. The signal due to residual CHCl₃ appearing at δ H 7.26 and (CH₃)₂SO appearing at δ H 2.5 and the central resonance of the CDCl₃ "triplet" appearing at δ C 77.0 and for (CD₃)₂SO "multiplet" appearing at δ C 39.0 were used to reference 1H and 13C NMR spectra, respectively. 1H NMR data are recorded as follows: chemical shift (δ) [multiplicity, coupling constant(s) J (Hz), relative integral] where multiplicity is defined as s=singlet; d=doublet; t=triplet; q=quartette; and m=multiplet or combinations of the above. Elemental analyses were determined using Manual Elemental Analyser Heraeus (Germany) and Automatic Elemental Analyser CHN Model 2400 Perkin Elmer (Waltham, MA, USA) at Microanalytical Centre, Faculty of Science, Cairo University, Egypt. All the elemental analyses results corresponded to calculated values within experimental error. Progress of reactions was monitored by thin-layer chromatography (TLC) using precoated TLC sheets with Ultraviolet (UV) fluorescent silica gel (Merck 60F254), and spots were visualised by iodine vapours or irradiation with UV light (254 nm). All chemicals were purchased from Sigma-Aldrich or Lancaster Synthesis Corporation (UK). Intermediates 6–8a–i were prepared according to reported procedure 47, 48.

2.1.1. General procedure for preparation of oxazolones (12a–h)

A mixture of N-(3,4,5-trimethoxybenzoyl)glycine (0.30 g, 1.10 mmol) and the appropriate aldehydes 8a–h (1.00 mmol) in acetic anhydride (1 ml) and fused sodium acetate (0.1 g, 1.2 mmol) was heated on an oil bath at 80 °C for 2 h. After cooling down at room temperature the mixture was allowed to stand for 24 h at 0 °C. The precipitate was filtered off and washed three times with ice-cooled ethanol (10 ml), and the product crystallised from ethanol.

2.1.1.1. 4-[[2-Methoxyquinolin-3-yl)methylen]-2–(3,4,5-trimethoxyphenyloxazol-5(4H)-one (12a).

Yellow solid, Yield (81%); m.p. 215–217 °C. IR (KBr): ν = 1776 (C=O), 1621 (C–N), 1599 (C=C) cm⁻¹. 1H NMR (400 MHz, CDCl₃) δ: 9.48 (s, 1H, Ar-H), 7.86–7.81 (m, 2H, Ar-H), 7.71–7.67 (m, 2H, Ar-H), 7.45–7.41 (m, 3H, Ar-H), 4.15 (s,
3H, OCH₃), 4.00 (s, 9H, 3 OCH₃) ppm. ¹³C NMR (100 MHz, CDCl₃): δ: 167.1, 163.9, 160.0, 153.5, 147.1, 143.1, 142.4, 134.6, 131.5, 128.9, 127.1, 125.2, 124.7, 123.6, 120.1, 118.5, 105.8, 61.1, 56.4, 54.1 ppm. MS (70 eV): m/z (%): 420 (7.79) [M⁺]; Anal. Calcd for C₂₅H₂₃N₃O₅: C, 65.71; H, 4.80; N, 6.66. Found: C, 65.64; H, 4.74; N, 6.71.

2.1.1.7. 4-[(3-Isopropoxy-2-methoxquinolin-3-yl)methylene]-2-(3,4,5-trimethoxyphenyl)-3,5-dihydro-4H-imidazol-4-one (12g). Yellow solid, yield (77%); m.p. 227–229°C. ¹H NMR (400 MHz, CDCl₃): δ ppm: 9.41 (s, 1H, Ar-H), 7.74–7.67 (m, 2H, Ar-H), 7.41 (s, 2H, Ar-H), 7.16–7.00 (m, 2H, Ar-H), 4.87–4.76 (m, 1H, OCH_3), 4.12 (s, 3H, OCH_3), 3.98 (s, 9H, 3 OCH₃), 1.45 (d, J = 4.0 Hz, 6H, 2 CH₃) ppm. ¹³C NMR (100 MHz, CDCl₃): δ: 167.4, 163.1, 161.2, 160.7, 153.5, 149.5, 142.9, 141.9, 133.2, 130.4, 126.4, 120.4, 119.9, 118.2, 115.7, 107.9, 105.7, 70.3, 61.3, 53.6, 53.9, 22.0 ppm. MS (70 eV): m/z (%): 478 (9.50) [M⁺]; Anal. Calcd for C₂₅H₂₃N₃O₅: C, 65.26; H, 5.48; N, 5.85. Found: C, 65.21; H, 5.45; N, 5.91.

2.1.1.8. 4-(3-Benzoyl)-2-(3,4,5-trimethoxyphenyl)-3,5-dihydro-4H-imidazol-4-one (12h). Pale yellow solid, yield (73%); m.p. 247–249°C. ¹H NMR (400 MHz, CDCl₃): δ ppm: 9.43 (s, 1H, Ar-H), 7.72 (t, J = 8 Hz, 2H, Ar-H), 7.51–7.35 (m, 6H, Ar-H), 7.28–7.26 (m, 2H, Ar-H), 7.14 (d, J = 1.0 Hz, 1H, Ar-H), 5.21 (s, 2H, OCH₂–), 4.12 (s, 3H, OCH₃), 3.98 (s, 9H, 3 OCH₃) ppm. ¹³C NMR (100 MHz, CDCl₃): δ: 167.4, 163.3, 161.9, 160.8, 153.5, 149.4, 142.9, 141.9, 136.2, 133.5, 130.2, 128.7, 128.3, 127.7, 124.4, 120.4, 120.3, 117.5, 116.0, 107.5, 105.6, 70.3, 61.3, 56.4, 53.9 ppm. MS (70 eV): m/z (%): 526 (7.30) [M⁺]; Anal. Calcd for C₂₅H₂₃N₃O₅: C, 68.43; H, 4.98; N, 5.35. Found: C, 68.39; H, 4.92; N, 5.41.

2.1.2. General procedure for preparation of (13a–h)

The appropriate oxazolones 12a–h (1 mmol) was stirred and heated under reflux in ethanol (10 mL) containing ammonium hydroxide (10 mL), and the reaction monitored by TLC. After completion of the reaction in 24 h, the solvent was concentrated and cooled, and the precipitate was filtered off and crystallised from ethanol.

2.1.2.1. 5-[(2-Methoxyquinolin-3-yl)methylene]-2-(3,4,5-trimethoxyphenyl)-3,5-dihydro-4H-imidazol-4-one (13a). Yellow solid, yield (81%); m.p. 230–232°C. IR (KBr, ν = 3222 (NH), 1709 (C=O), 1642 (C=N), 1615, 1585 (C=C) cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆): δ ppm: 12.22 (s, 1H, exch., NH), 9.69 (s, 1H, Ar-H), 8.03 (d, J = 8.0 Hz, 1H, Ar-H), 7.80–7.70 (m, 2H, Ar-H), 7.59 (s, 2H, Ar-H), 7.49 (t, J = 8.0 Hz, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 4.11 (s, 3H, OCH₃), 3.94 (s, 6H, 2 OCH₃3), 3.80 (s, 3H, OCH₃) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ: 187.4, 165.0, 164.2, 161.3, 157.9, 153.5, 146.7, 140.9, 132.2, 128.9, 126.7, 125.2, 123.9, 118.4, 108.7, 104.2, 60.3, 56.3, 54.1 ppm. MS (70 eV): m/z (%): 419 (5.40) [M⁺]; Anal. Calcd for C₂₅H₂₃N₃O₅: C, 65.86; H, 5.05; N, 10.02. Found: C, 65.81; H, 4.99; N, 10.09.

2.1.2.2. 5-[(2-Methoxy-6-quinolin-3-yl)methylene]-2-(3,4,5-trimethoxyphenyl)-3,5-dihydro-4H-imidazol-4-one (13b). Yellow solid, yield (81%); m.p. 223–225°C. ¹H NMR (400 MHz, DMSO-d₆): δ ppm: 12.18 (s, 1H, exch., NH), 9.55 (s, 1H, Ar-H), 7.74–7.54 (m, 5H, Ar-H), 7.26–7.24 (d, J = 6.6 Hz, 1H, Ar-H), 4.07 (s, 3H, OCH₃), 3.93 (s, 6H, OCH₃), 3.80 (s, 3H, OCH₃), 2.47 (s, 3H, CH₃) ppm. ¹³C NMR (100 MHz, DMSO-d₆): δ: 172.0, 162.2, 156.0, 153.7, 144.6, 142.6, 142.4, 141.2, 134.4, 133.4, 128.1, 126.8, 125.4, 123.2, 119.3, 116.2, 110.0, 106.2, 60.7, 56.8, 54.2, 21.3 ppm. MS (70 eV): m/z (%): 433 (5.60) [M⁺]; Anal. Calcd for C₂₅H₂₃N₃O₅: C, 66.50; H, 5.35; N, 9.69. Found: C, 66.41; H, 5.28; N, 9.75.
2.1.2.3. 5-[[2-Methoxy-7-methylquinolin-3-yl]methylene]-2-[[3,4,5-trimethoxy-3,5-dihydro-4H-imidazol-4-one (13c). Yellow solid, Yield (84%); m.p. 236–238°C. IR (KBr): v = 3215 (NH), 1711 (C=O), 1639 (C=N), 1590 (C=C) cm⁻¹. 1H NMR (400 MHz, DMSO-d6): δ: 12.17 (s, 1H, exch., NH), 9.63 (s, 1H, Ar-H), 7.92 (d, J = 8.0 Hz, 1H, Ar-H), 7.58 (s, 3H, Ar-H), 7.32 (d, J = 8.0 Hz, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 4.09 (s, 3H, OCH₃), 3.94 (s, 6H, 2OCH₃), 3.80 (s, 3H, OCH₃), 2.49 (s, 3H, CH₃) ppm. 13C NMR (100 MHz, DMSO-d6): δ: 162.6, 162.5, 160.3, 153.9, 142.2, 141.5, 129.1, 127.1, 126.3, 123.6, 118.3, 115.8, 105.6, 60.8, 56.5, 54.2, 22.0 ppm. MS (70 eV): m/z (%): 433 (8.40) [M⁺]; Anal. Calcd for C₂₄H₂₃N₃O₆: C, 64.13; H, 5.10; N, 9.29.

2.1.2.4. 5-[[2-Methoxy-8-methylquinolin-3-yl]methylene]-3,5-dihydro-4H-imidazol-4-one (13d). Yellow solid, Yield (77%); m.p. 259–261°C. 1H NMR (400 MHz, DMSO-d6): δ: 12.16 (s, 1H, exch., NH), 9.62 (s, 1H, Ar-H), 7.96–7.94 (d, J = 8.0 Hz, 1H, Ar-H), 7.58–7.28 (m, 10H, Ar-H), 5.30 (s, 2H, OCH₂), 4.09 (s, 3H, OCH₃), 3.94 (s, 6H, 2OCH₃), 3.79 (s, 3H, OCH₃) ppm. 13C NMR (400 MHz, DMSO-d6): δ: 163.9, 161.6, 158.5, 153.5, 148.8, 140.7, 140.3, 136.6, 130.2, 128.0, 127.8, 118.9, 118.5, 117.8, 107.2, 105.9, 104.0, 69.7, 60.4, 56.3, 54.0 ppm. MS (70 eV): m/z (%): 525 (6.75) [M⁺]; Anal. Calcd for C₂₉H₂₇N₃O₆: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.51; H, 5.13; N, 8.05.

2.2. Biochemical evaluation of activity

All biochemical assays were performed in triplicate on at least three independent occasions for the determination of mean values.

2.2.1. Cell culture

The four human tumour cell lines MCF-7, HCT-116, HL-60 and HeLa used in this study were obtained from the VACSERA (Giza, Egypt) culture cell unit that were originally acquired from ATCC (Manassas, VA, USA). All the human tumour cell lines were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% foetal bovine serum, 2 mM L-glutamine and 100 μg/mL penicillin/ streptomycin. Cells were maintained at 37°C in 5% CO₂ in a humidified incubator. All cells were sub-cultured 3 times/week by trypsinisation using TrypLE Express (1X).

2.2.2. Cell viability assay

The quinoline compounds were evaluated for antiproliferative effect using the MTT viability assay of four cancer cell lines (MCF-7, HCT-116, HL-60 and HeLa) and normal breast cells MCF-10A to calculate the relative IC₅₀ values for each compound. Cells were seeded in triplicate in 96-well plates at a density of 10⁴ cells/ml in a total volume of 200 μl per well. 0.1% of DMSO was used as a vehicle control. Following, the cells were treated with 2 μl test compound (from stock solutions in ethanol) to furnish the concentration range of study, 1 nM to 50 μM, and re-incubated for a further 72 h. The culture medium was then removed, and the cells washed with 100 μL phosphate buffered saline (PBS) and 50 μL MTT added, to reach a final concentration of 1 mg/mL. Cells were incubated for 2 h in darkness at 37°C. Solubilisation was begun through the addition of 200 μL DMSO, and the cells maintained at room temperature in darkness for 20 min to ensure thorough colour diffusion before reading the absorbance. Plates were incubated for 72 h at 37°C in 5% CO₂. Results were expressed as percentage viability relative to vehicle control (100%). Dose response curves were plotted and IC₅₀ values (concentration of drug resulting in 50% reduction in cell survival) were obtained using the commercial software package Prism (GraphPad Software, Inc., La Jolla, CA, USA). All the experiments were repeated in at least three independent experiments.

2.2.3. Tubulin polymerisation assay

The assembly of purified bovine tubulin was monitored using a kit, BK006, purchased from Cytoskeleton Inc., (Denver, CO, USA). The assay was carried out in accordance with the manufacturer’s
instructions using the standard assay conditions. Briefly, purified (>99%) bovine brain tubulin (3 mg/mL) in a buffer consisting of 80 mM PIPES (pH 6.9), 0.5 mM MgCl₂, 2 mM EGTA, 1 mM GTP and 10% glycerol was incubated at 37°C in the presence of either vehicle (2% (v/v) ddH₂O), CA-4, or the quinoline compounds. Light is scattered proportionally to the concentration of polymerised microtubules in the assay. Therefore, tubulin assembly was monitored turbidimetrically at 340 nm in a SpectraMax 340 PC spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). The concentration that inhibits tubulin polymerisation by 50% (IC₅₀) was determined using area under the curve (AUC). The AUC of the untreated controls were considered as 100% polymerisation. The IC₅₀ value for each compound was computed using GraphPad Prism Software.

2.2.4. Colchicine site competitive binding assay
The affinity of compounds 12c to colchicine binding site was determined using Colchicine Site Competitive Assay kit CytoDYNAMIX Screen15 (Cytoskeleton, Inc., Denver, CO, USA) using the standard protocol of the manufacturer to determine Ki. Biotin-labelled tubulin (0.5 μg) in 10 μL of reaction buffer was mixed with [3H]colchicine (0.08 μM, PerkinElmer, Waltham, MA) and the test compounds (positive control colchicine, negative control vinblastine, G-1, fluorescent G-1, or 2-ME) in a 96-well plate (final volume: 100 μL). After incubating for 2 h at 37°C with gentle shaking, streptavidin-labelled yttrium SPA beads (80 μg in 20 μL reaction buffer, PerkinElmer, Waltham, MA) were added to each well and incubated for 30 min at 4°C. The plates were then read on a scintillation counter (Packard Instrument, Topcount Microplate Reader) and the percentage of inhibition was calculated.

2.2.5. Cell cycle analysis
MCF-7 cells were seeded at a density of 1 x 10⁵ cells/well in 6-well plates and treated with CA-4 (50 nM) and compound 12c (50 and 250 nM) for 24, 48 and 72 h. The cells were collected by trypsinisation and centrifuged at 800 x g for 15 min. Cells were washed twice with ice-cold PBS and fixed in ice-cold 70% ethanol overnight at -20°C. Fixed cells were centrifuged at 800 x g for 15 min and stained with 50 μg/mL of PI, containing 50 μg/mL of DNase-free RNase A, at 37°C for 30 min. The DNA content of cells (10,000 cells/experimental group) was analysed by flow cytometer at 488 nm using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) and all data were recorded and analysed using the CellQuest Software (Becton-Dickinson).

2.2.6. Annexin V/PI apoptotic assay
Apoptotic cell death was detected by flow cytometry using Annexin V and propidium iodide (PI). MCF-7 Cells were seeded in 6 well plated at density of 1 x 10⁵ cells/mL and treated with vehicle (0.1% (v/v) EtOH), positive control (CA-4) or compound 12c (50 and 250 nM) for 24, 48 and 72 h. Cells were then harvested and prepared for flow cytometric analysis. Cells were washed in 1X binding buffer (20X binding buffer: 0.1 M HEPES, pH 7.4; 1.4 M NaCl; 25 mM CaCl₂ diluted in dH₂O) and incubated in the dark for 30 min on ice in Annexin V-containing binding buffer [1:100]. Cells were then washed once in binding buffer and then re-suspended in PI-containing binding buffer [1:1000]. Samples were analysed immediately using the BD accuri flow cytometer and prism software for analysis the data. Four populations are produced during the assay Annexin V and PI negative (Q4, healthy cells), Annexin V positive and PI negative (Q3, early apoptosis), Annexin V and PI positive (Q2, late apoptosis) and Annexin V negative and PI positive (Q1, necrosis).

2.2.7. Evaluation of expression levels of anti-apoptotic proteins bcl-2, pro-apoptotic proteins bax and caspase 9
The level of the anti-apoptotic marker and apoptotic marker BAX were assessed using Bcl-2 Elisa kit and human Bax ELISA Kit purchased from Zymed laboratories, invitrogen and Cloud-Clone Crop. (Katy, TX, USA), respectively, following the manufacturer’s instructions. Briefly, Treated MCF-7 cell lysate with 250 nM of compound 12c were prepared, and equal amount of cell lysates were loaded and propped with specific antibodies. The samples were measured and analysed at 450 nm in ROBONEK P2000 ELISA reader. In Vitro Caspase-9 Activation Assay was performed using human active caspase-9 Invitrogen EIA kit according to the manufacturer’s instructions. Compound 12c at concentrations of 50 and 250 nM and CA-4 (50 nM) were prepared in dH₂O up to a final volume of 50 μL/well followed by addition of 5 μL of active caspase-9. Following, the cells were mixed and 50 μL of the Master Mix was added to each well and allowed to react at 37°C for 1 h. The fluorescence intensity of the test samples was recorded and analysed in a fluorescence plate reader at 405 nm excitation and 505 nm emissions. All experiments were conducted in triplicates.

2.2.8. Colony formation assay
MCF-7 cells (600 cells per well) were seeded in 6-well plates and incubated for 24 h before being then treated with different doses of the compound 12c (50 and 250 nM) for 14 days. Following, the cells were washed with PBS twice and subsequently fixed with 4% paraformaldehyde and stained with 0.05% crystal violet for 30 min. Finally, cells were visualised using an inverted microscope.

2.2.9. Wound healing assay
MCF-7 were grown in 6-well plates for 24 h, and scratches were made using pipette tip and washed with PBS to remove non-adherent cell debris. Subsequently, the cells were treated with different concentrations of 12c for 24 h. The migrations across the wound area were photographed under a phase contrast microscope.

2.2.10. Measurement of mitochondrial depolarisation effect (∆Ψₘ) and ROS levels in cells
Mitochondrial membrane potential (∆Ψₘ) was measured by flow cytometry with DiOC2(3) staining and additional labelling with an annexin V conjugate. After treatment with compound 12c (50 and 250 nM) and CA-4 (50 nM), cells were stained with DiOC2(3) dye for 30 min in the incubator, then harvested and washed with PBS. DiOC2(3)-stained cells were resuspended with 1X annexin binding buffer, followed by addition of annexin V conjugate and incubated at 37°C for 15 min. The data from the flow cytometry were analysed by Cell Quest software. Production of intracellular reactive oxygen species (ROS) was measured using 2′,7′-dichlorofluorescin diacetate (H₂DCFDA) dye. MCF-7 cells were seeded and treated either with vehicle (0.1% DMSO) or with compound 12c (50 and 250 nM) or CA-4 (50 nM) for 6, 12 and 24 h. H₂O₂ was used as a positive control. The amount of ROS generated was estimated after 2h of selected compound treatment. The cells were collected by centrifugation and washed twice with PBS. Cells were then incubated with DCFDA dye (25 μM) in dark at 37°C for 1 h.
Fluorescence spectra (510 – 600 nm) were monitored using an excitation wavelength of 488 nm.53,54.

3. Results and discussion

3.1. Design and chemistry

The natural product, Combretastatin A-4 (CA-4; Figure 1) exhibits significant antiproliferative activities against several tumour cells by binding to the colchicine site of tubulin to inhibit the protein polymerisation20. However, the cis double bond of CA-4 has a propensity to isomerise into the inactive trans configuration, leading to reduction in the molecule’s pharmacologic activity. Several structural modifications of the CA-4 pharmacophore have subsequently been undertaken to overcome this disadvantage, e.g. replacing the cis double bond with a heterocycle, oxadiazole, isoxazole and imidazole, resulting in compounds, such as 1, 2 and 3 respectively (Figure 1)27–32. In this work, we undertook a rational design approach of introducing chalcone system (ring C) in the form of either oxazolone or imidazolone between the two rings A and B, as well as isosterically replace ring B with quinolone. Specifically, the rigidity of the molecules was increased by introducing 1,3-oxazol-5-ones and 1,3-imidazol-4-ones to the cis-olefinic bond of CA-4, which we anticipate would create a desirable conformational and configurational restriction to prevent isomerisation of CA-4 into the inactive trans-isomer, as well as improve on the anticancer activities of these compounds since chalcones are well known for their anticancer properties 55,56. The second design step involves varying the electronic substituents effect on the quinolyl moiety (ring B), while maintaining the natural active compound 3,4,5-trimethoxyphenyl moiety, which we anticipate will increase the potency of these compounds.

The syntheses of the proposed quinoline compounds 12a–h (oxazolones) and 13a–h (imidazolones) (Table 1) are shown in Schemes 1 and 2 and involve two core structural components: (i) 2-methoxyquinolyl-3-carbaldehyde nucleus 8a–h, and (ii) 3,4,5-trimethoxyphenyl moiety 11. A concise (three-step) synthesis was used for the synthesis of the first core structure, 2-

![Scheme 1](image_url)
methoxyquinoline-3-carbaldehyde derivatives 8a–h, as shown in Scheme 1. The synthesis was initiated with acetylation of the starting aniline derivatives 5a–h using acetic anhydride and glacial acetic acid at 0°C. The produced amides 6a–h were subjected to Vilsmeier–Haack reaction to give the corresponding quinoline-3-aldehyde derivatives 7a–h. Addition of methoxy substituent to 7a–h to give 8a–h was achieved through the use of sodium methoxide at 40°C in methanol[47,48]. The synthesis of the second core 3,4,5-trimethoxyphenyl moiety 11 started with acylation of the acid 9 under highly acidic condition using SOCl₂ to give acyl benzotriazole 10 (Scheme 2)[37,38]. Following, treatment of the acyl benzotriazole 10 with glycine in aqueous acetonitrile gave the acyl glycine 11. Condensation of 11 with the appropriate quinoline aldehydes 8a–h in the presence of acetic anhydride and catalytic amount of sodium acetate resulted in the formation of the oxazolones 12a–h. Aminolysis of 12a–h via condensation reaction with ammonia led to the formation of the imidazolones 13a–h. It seems the nucleophilic ammonia attacks the carbonyl group of the oxazolone ring, followed by immediate intramolecular condensation and cyclisation to give the imidazolones 13a–h. In summary, two classes of compounds, 12a–h (oxazolones) and 13a–h (imidazolones) were synthesised and used for further functional and biological studies.

Table 2. Inhibition of Tubulin Polymerisation and Colchicine Binding by quinoline compounds and CA-4.

| Compound number | IC₅₀ (µM) | 1 µM drug | 5 µM drug |
|-----------------|-----------|------------|-----------|
| 12a             | 13.98     | nd         | nd        |
| 12c             | 2.12      | 79 ± 2     | 87 ± 1    |
| 12e             | 2.26      | nd         | nd        |
| 12g             | 8.23      | nd         | nd        |
| 13c             | 20.29     | nd         | nd        |
| 13e             | 1.48      | nd         | nd        |
| CA-4            | 2.17      | 86 ± 0.9   | 97 ± 2    |

Inhibition of tubulin polymerisation. Tubulin was at 10±µM. Inhibition of [²H] colchicine binding. Tubulin and colchicine were at 1 and 5 µM concentrations, respectively.

3.2. Biological results and discussion

3.2.1. In vitro antiproliferative activities

All the synthesised compounds (with CA-4 as a positive reference) were evaluated for their antiproliferative activities using MTT assay with four different cancer cell lines — MCF-7 breast adenocarcinoma, HL-60 leukaemia, HCT-116 colorectal carcinoma, and HeLa cervical adenocarcinoma. As shown in Table 1, most of the compounds demonstrated moderate to highly potent antiproliferative activities. In the oxazolone analogues (12a–h), compound 12a without any substituent on the quinoline ring was the least active when compared with quinoline ring substituted compounds. The relative position of the substituent on the quinoline ring also seemed to be critical for antiproliferative activity. Compound 12c with methyl group at the 7-position ring displayed impressive non-selective potency in nanomolar range against HL-60, MCF-7, HCT-116 and HeLa cell lines with IC₅₀ of 0.019, 0.010, 0.022 and 0.042 µM, which compared to 0.076, 0.019, 0.026 and 0.064 µM for CA-4, respectively. In contrast, both the 6-CH₃ analog (12b) and 8-CH₃ analog (12d) were 3- to 15-fold less active than 12c. The nature of the substituents on the quinoline ring of the oxazolone compounds was also found to significantly influence the biological activity. For example, replacement of the methyl group in 12b and 12c with a stronger electron-releasing methoxy group yielded compounds 12e and 12f, respectively, which resulted in better antiproliferative activities. The methoxy-containing compound 12e was 2.7- to 13-fold more active than the methyl-containing compound 12b with the four cancer cell lines (IC₅₀ of 0.068, 0.056, 0.031 and 0.010 µM in HL-60, MCF-7, HCT-116 and HeLa cancer cell lines, respectively). Compound 12f had a similar effect as 12c against MCF-7 and HCT-116 cells (0.052 and 0.066 µM, respectively), but with reduction in activity against the other two cell lines, HL-60 and HeLa (0.352 and 0.138 µM, respectively). Introducing larger substituents at the quinoline ring as in 12g (7-tert-butyl) and 12h (7-benzyloxy) led to a dramatic decrease in activity compared to their corresponding analog 12f (7-methoxy). Summarily, adding smaller and/or polar groups to the quinoline ring of the oxazolone resulted in significant improvement in the antiproliferative activity.
The imidazolones (Compound 13a–h) also resulted in impressive antiproliferative activity with IC_{50} values ranging from 0.04–8.21 μM in all four cell lines. In general, the imidazolones showed similar antiproliferative activities as the oxazolones (Table 1), which could be due to similar electronic effects of the oxazolone and imidazolone rings. Like the oxazolone, lack of substituent
on the quinolone ring as in compound 13a led to reduction in activity, with IC\textsubscript{50} values of more than 1 \( \mu \)M in all four cell lines, similar to the results obtained with the oxazolone derivative 12a.

Methyl substitution on the quinoline ring, e.g. 6-CH\textsubscript{3} 13b, 7-CH\textsubscript{3} 13c and 8-CH\textsubscript{3} 13d led to potent activity in submicromolar range in all four cancer cell lines.

The position of the methoxy substituent on the quinoline heterocycle also influenced the antiproliferative activity of the

Figure 4. (A) Effect of compound 12c at different time points on apoptosis in MCF-7 cells analysed by flow cytometry after double staining of the cells with Annexin-V-FITC and PI. MCF-7 cells treated with 50 and 250 nM of compound 12c and 50 nM of CA-4 for 24 h, 48 h and 72 h and collected and processed for analysis. (B) Quantitative analysis of apoptosis. Values represent the mean ± SEM for three independent experiments. Statistical analysis was performed using two-way ANOVA (\( ^* p < 0.05 \), \( ^{**} p < 0.01 \), \( ^{***} p < 0.001 \)).
compounds against the cancer cell lines. For example, the antiproliferative activity of 6-methoxy-substituted \(13e\) was better than its analog 7-methoxy-substituted \(13f\) against MCF-7, HCT-116 and HeLa cells with IC\(_{50}\) values of 0.042, 0.085 and 0.062 \(\mu\)M, which compare to 0.092, 0.187 and 0.101 \(\mu\)M for \(13f\), respectively. However, in HL-60, \(13e\) exhibited less antiproliferative activity with IC\(_{50}\) value of 0.272 \(\mu\)M. In a similar trend as the oxazolone derivatives, bulky substituents on the quinoline ring \(13g\) (7-tert-butyl) and \(13h\) (7-benzyloxy) resulted in drastic decrease in activity in all four cancer cell lines with 14- to 125-fold loss in potency compared to their corresponding \(13f\) (7-methoxy containing) compound.

In summary, both oxazolone and imidazolone compounds displayed potent antiproliferative effects, strengthening our hypothesis that nitrogen-containing heterocycles, such as quinoline, are beneficial surrogates for the ring B of CA-4. The different biological activities of the compounds are likely the result of differences in their mode of interaction with the colchicine binding site. Due to its excellent antiproliferative activity, compound \(12c\) was studied in more details as described below.

### 3.2.2. In vitro inhibition of tubulin polymerisation and colchicine binding

Trimethoxyphenyl (TMP) containing stilbenoid derived compounds, such as colchicine, resveratrol and CA-4 bind to tubulin at the colchicine binding site, resulting in inhibition of microtubule polymerisation\(^{59,60}\). To confirm whether the quinoline compounds similarly target the tubulin-microtubule system, representative quinoline compounds, including four oxazolone analogues (\(12a\), \(12c\), \(12e\) and \(12g\)) and two imidazolone analogues (\(13c\) and \(13e\)), as well as the reference compound CA-4, were evaluated for their antitubulin polymerisation activities and the results presented in Table 2. The methyl and methoxy substituted oxazolone compounds \(12c\) and \(12e\), respectively strongly inhibited tubulin assembly with IC\(_{50}\) of 1.21 and 2.26 \(\mu\)M, respectively compared to that of CA-4 (IC\(_{50}\) of 2.17 \(\mu\)M), while the

---

**Figure 5.** Dose response curve for (A) Compound \(12c\) and (B) CA-4 on the proliferation of breast cancer MCF-7 and normal breast MCF-10A cells. Cells were grown in 96-well plates and treated with serial concentrations of compound \(12c\) or CA-4 for 72 h. Cell viability was expressed as percentage of vehicle control [ethanol 1% (v/v)] treated cells and was measured by MTT assay (average of three independent experiments).

**Figure 6.** Effect of compound \(12c\) on the expression (A) anti-apoptotic protein Bcl2, (B) pro-apoptotic protein BAX and (C) Caspase 9 in MCF-7 cells.
unsubstituted analogue 12a (IC_{50} of 13.98 μM) and tert-butyl ana-
log 12g (IC_{50} of 8.23 μM) were 6- and 4-fold less active than CA-4.
The imidazolone compound 13e with IC_{50} of 1.48 μM also showed
very potent tubulin polymerisation inhibition compared to CA-4.
The methyl analogue 13c was inactive in the tubulin polymerisa-
tion assay (IC_{50} of 20.29 μM), and is 16-fold less active compared
to its corresponding oxazolone derivative 12c, which is in agree-
ments with the poor cell growth inhibitory activity of 13c com-
pared to 12c.

Compound 12c was also examined at two different concentra-
tions (1 and 5 μM) for its ability to compete with colchicine for
binding to tubulin using a [3H] colchicine binding assay. Compound 12c
strongly inhibited colchicine binding to tubulin by 79% and 87% at 1 and 5 μM respectively, which compares with 86% and 97% inhibition by CA-4, respectively. These results sug-
gest that compound 12c is involved in tubulin polymerisation
inhibition through the colchicine-binding site.

3.2.3. Cell cycle analysis
Induction of cell cycle arrest at G2/M phase is strongly accompa-
nied with tubulin polymerisation inhibition. It is well established
that CA-4 arrests cell cycle at G2/M phase^{61–63}. To further gain
insight into compound 12c potent antiproliferative activity, cell
cycle analysis of MCF-7 cells was performed at two concentrations
of 50 nM and 250 nM and at different time points of 0, 24, 48 and
72 h. Figure 3(A) clearly demonstrates that 12c caused a signifi-
cant arrest in G2/M phase and apoptosis in a dose- and time-
dependent manner. After 48 h, the percentage of G2/M phase
arrested cells were 28.4% and 38.3% at 50 nM and 250 nM,
respectively compared to 9.2% of untreated cells (Figure 3(B)).
Moreover, there was an increase in the number of cells in G2/M
phase after 72 h (33.0% and 40.8% at 50 nM and 250 nM, respect-
ively) with a concomitant decrease of cells in G_0/G_1 phase (40.3%
and 29.8% at 50 nM and 250 nM, respectively) compared to the
control (57.3%). In a comparable finding, CA-4 (50 nM) also signifi-
cantly arrested G2/M phase at 24, 48 and 72 h (40.3%, 43.8% and
47.7%, respectively). Accordingly, a concomitant decrease of MCF-
7 cells was detected in G0 phase (Figure 3(C)). Furthermore, com-
 pound 12c induced a gradual increase in apoptosis (16.2%, 23.4%
and 32.7%) at 250 nM as the proportion of cells in the sub-G1
phase increased at 24, 48 and 72 h, respectively compared to
untreated cells (1.5%) (Figure 3(D)). Similarly, 23.5%, 31.1% and
37.4% increase in apoptosis was observed for CA-4 at 24, 48 and
72 h, respectively. These findings are in agreement with previously
reported for a series of related quinoline analogues, which signifi-
cantly induced apoptosis and G2/M cycle arrest in MCF-7
cells^{23,42,46,64}.

3.2.4. Cell apoptosis
We investigated whether cell death induced by compound 12c
treatment was related to apoptosis using the Annexin-V/PI double
staining flow cytometric assay (Figure 4(A,B)). MCF-7 cells were
treated with three different concentrations (0, 50 and 250 nM) of
compound 12c at different time points (24, 48 and 72 h).
Compound 12c caused a significant accumulation of annexine-V positive cells and induced both early and late apoptosis in a dose- and time-dependent manner compared to the untreated cells. As shown in Figure 4(B), when the cells were treated with 12c (0 and 250 nM) or CA-4 (50 nM) for 48 h, the percentage of Annexin V-staining positive cells significantly increased from 1% in untreated cells to 15%, 21% and 29% respectively. The percentage of early and late apoptotic cells in the presence of 12c increased after 72 h to 17.6% and 29.3% at 1 and 5 µM respectively when compared to the untreated cells (2%). Based on the cell cycle arrest and apoptosis findings (Figure 3(B–D)), it appears that compound 12c could efficiently induce apoptosis cell death in MCF-7 cells in a dose- and time-dependent manner.

3.2.5. Assessment of toxicity to non-tumorigenic human cells
To assess the cytotoxicity and selectivity of 12c towards cancer cells, normal epithelial breast MCF-10A cell viability study was carried out. As shown in Figure 5(A), the IC50 value of 12c was more than 50 µM in MCF-10A cells, which was significantly higher than the IC50 values of 19, 10, 22 and 42 nM in MCF-7, HL-60, HCT-116 and HeLa cancer cell lines, respectively. Remarkably, 12c was found to be less toxic in normal MCF-10A (IC50 > 50 µM) when compared to CA-4 (IC50 = 6.1 µM) (Figure 5(B)), suggesting 12c to have better selective toxicity against cancer cells.

3.2.6. Expression of the apoptotic proteins in MCF-7 cell lines
The previous data clearly demonstrate that 12c is an effective anti-mitotic quinoline compound in MCF-7 cell lines. Herein, the effect of 12c on the expression of apoptosis pathway markers, Bcl-2 anti-apoptotic protein and Bax pro-apoptotic protein was investigated. MCF-7 cells treated with 12c at 250 nM for 48 h decreased the expression level of the anti-apoptotic protein Bcl-2, and correspondingly up-regulated the expression of the pro-apoptotic protein Bax (Figure 6(A,B)).

Activation of caspases initiates apoptosis, and in particular caspase-9 is considered an important effector caspase responsible for

Figure 8. (A) Inhibition of the migration of MCF-7 cells treated with compound 12c for 48 h in the wound healing assay. (B) Quantitative analysis of wound closure rate and was calculated as mean ± SEM for three independent experiments. Statistical analysis was performed using one-way ANOVA-Bonferroni post-hoc test (**p < 0.001).
programmed cell death apoptosis activated by CA-4\textsuperscript{61,65,66}. The amount of activated caspase-9 was examined in MCF-7 cells treated with 12c. As observed from Figure 6(C), compound 12c at 50 and 250 nM produced about 8- and 16-fold increases in caspase-9 activation respectively when compared to 14-fold for CA-4 (50 nM). This finding confirms that compound 12c like CA-4 enhanced the rate of apoptosis in MCF-7 cell through caspase-9 activation.

3.2.7. Inhibition of colony formation

Colony formation assay is one of the effective techniques for the determination of long-term cell proliferation upon anticancer drug exposure. The inhibitory potential of 12c on MCF-7 cells colony formation is displayed in Figure 7. Compound 12c suppressed the clonogenic formation potential of MCF-7 cells in a dose depend-ent manner when compared to CA-4.
Mitochondria membrane potential plays a crucial role in the propagation of apoptosis. Specifically, loss of mitochondrial membrane potential is characteristic of early stage of apoptosis\(^{(69,71)}\). To confirm whether compound 12c could decrease the MMP of MCF-7 cancer cells, MMP was monitored by the fluorescence of the dye DiOC\(_2\)(3). MCF-7 cells treated with 12c at 50 and 250 nM exhibited significant decrease in MMP in a dose- and time-dependent manner (Figure 9(A)). This depletion in MMP was associated with an increase of annexin-V positive early apoptotic cells. Maximum decrease in MCF-7 MMP was detected after 24 h treatment with 12c in which the percentage of apoptotic cells increased from 1.1% to 24.9% and 31.3% at 50 and 250 nM, respectively (Figure 9(B)). This indicates that compound 12c induces mitochondrial dysfunction in MCF-7, which eventually triggered apoptotic cell death. These results are in agreement with previously reported CA-4 analogues study that were shown to cause apoptosis through the mitochondrial pathway\(^{(68,72,73)}\).

### 3.2.9. Mitochondrial membrane potential

Mitochondria membrane potential plays a crucial role in the propagation of apoptosis. Specifically, loss of mitochondrial membrane potential \(\Delta \psi_{\text{mit}}\) (MMP) is characteristic of early stage of apoptosis\(^{(69,71)}\). To confirm whether compound 12c could decrease the MMP of MCF-7 cancer cells, MMP was monitored by the fluorescence of the dye DiOC\(_2\)(3). MCF-7 cells treated with 12c at 50 and 250 nM exhibited significant decrease in MMP in a dose- and time-dependent manner (Figure 9(A)). This depletion in MMP was associated with an increase of annexin-V positive early apoptotic cells. Maximum decrease in MCF-7 MMP was detected after 24 h treatment with 12c in which the percentage of apoptotic cells increased from 1.1% to 24.9% and 31.3% at 50 and 250 nM, respectively (Figure 9(B)). This indicates that compound 12c induces mitochondrial dysfunction in MCF-7, which eventually triggered apoptotic cell death. These results are in agreement with previously reported CA-4 analogues study that were shown to cause apoptosis through the mitochondrial pathway\(^{(68,72,73)}\).

### 3.2.8. Wound healing assay

Migration and motility of cancer cells are considered as critical factors in tumour progression and metastasis\(^{(67,68)}\). In order to investigate the effect of compound 12c on the migration of MCF-7, wound healing assay was performed. As illustrated in Figure 8(A,B), the untreated cells migrated to the scraped area while in 12c-treated wells, cell migration was significantly inhibited in a dose-dependent manner. This significant difference in the wound area confirms that 12c suppressed MCF-7 cell migration, an important event in tumour metastasis.

### 3.2.10. Intracellular reactive oxygen species (ROS) production

The dissipation of mitochondrial potential is strongly associated with mitochondrial production of reactive oxygen species (ROS)\(^{(71,73)}\). The production of ROS after 12c treatment at 50 and 250 nM, as well as CA-4 (50 nM) with hydrogen peroxide H\(_2\)O\(_2\) was followed with 2,7-dichlorofluorescin diacetate (H\(_2\)-DCFDA). As shown in Figure 10, after 24 h of 12c treatment, the levels of ROS in MCF-7 cells were 22.7 and 26.6% at 50 and 250 nM, respectively. The level in untreated MCF-7 cells was 1.0%, while it increased only to 18.3% in CA-4-treated cells. This result along with the significant loss of mitochondrial membrane potential above clearly suggests that compound 12c induced apoptosis via the mitochondrial pathway.

### 4. Conclusion

In this study, we designed, synthesised and evaluated two classes of novel quinoline compounds combretastatin A-4 derivatives as potential inhibitors of tubulin polymerisation. Several other studies have also reported derivatisation of the CA-4 pharmacophore with varying success\(^{(27-32)}\). Unlike the previous compounds, we for the first time introduced a chalcone system, including oxazolones and imidazolones to the cis bond of CA-4 to give more rigidity to the required active conformation. The chalcone system is well known for its anticancer activities. Our design also kept the essential natural trimethoxophenyl pharmacophore (found in CA-4), while varying the electronic substituents effect on the quinolyl moiety (ring B) that were expected to enhance the potency of the compounds. Most of the compounds showed significant and, in some instances, comparable antiproliferative activities against different cancer cell lines as the previously studied combretastatin A-4 compound, CA-4. One of the most promising compound 12c showed potent anti-proliferative activities against HL-60, MCF-7, HCT-116 and HeLa cancer cell lines with IC\(_{50}\) values of 0.019, 0.010, 0.022 and 0.042 \(\mu\)M, respectively, and simultaneously low cytotoxicity towards MCF-10A non-cancer cells. The microtubule polymerisation inhibitory effect of 12c was confirmed with an in vitro tubulin polymerisation and colchicine inhibition assays. Compound 12c effectively block the G\(_2\)/M phase at the cell cycle and induce MCF-7 cell apoptosis together with significant change of Bax/Bcl expression ratio indicating involvement of mitochondrial apoptosis pathway. Further cellular mechanistic studies confirmed that 12c inhibited MCF-7 cell migration and colony formation. In conclusion, these results highlight our novel quinoline compounds and particularly 12c as promising anti-tubulin agent for the treatment of MCF-7 breast cancer cells. Moreover, the results point to a direction for rational development of potent tubulin polymerisation inhibitors for the treatment of cancer.

### Acknowledgements

The authors gratefully acknowledge DSR technical and financial support.

### Disclosure statement

The authors declare no competing interests.

### Funding

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant number [RG-18-166-41]. The authors, therefore, gratefully acknowledge DSR technical and financial support.

### ORCID

Tarek S. Ibrahim \(\underline{http://orcid.org/0000-0002-3049-4617}\)
Azizah M. Malebari \(\underline{http://orcid.org/0000-0003-4178-0341}\)
Ehab S. Taher \(\underline{http://orcid.org/0000-0002-1091-2885}\)
Abdelsattar M. Omar \(\underline{http://orcid.org/0000-0002-9825-3465}\)
Yaseen A. M. Elshaier \(\underline{http://orcid.org/0000-0003-4332-6345}\)
References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.

2. Decosterd LA, Widmer N, Zaman K, et al. Therapeutic drug monitoring of targeted anticancer therapy. Biomark Med 2015;9:887–93.

3. La Regina G, Coluccia A, Naccarato V, Silvestri R. Towards modern anticancer agents that interact with tubulin. Eur J Pharm Sci 2019;131:58–68.

4. Stec-Martyna E, Ponassi M, Miele M, et al. Structural comparison of the interaction of tubulin with various ligands affecting microtubule dynamics. Curr Cancer Drug Targets 2012;12:658–66.

5. Devi Tangutur A, Kumar D, Vamsi Krishna K, Kantevari S. Microtubule targeting agents as cancer chemotherapeutics: An overview of molecular hybrids as stabilizing and destabilizing agents. Curr Top Med Chem 2017;17:2523–37.

6. Kavallaris M. Microtubules and resistance to tubulin-binding agents. Nat Rev Cancer 2010;10:194–204.

7. Coullup SK, Georg Gl. Revisiting microtubule targeting agents: A-tubulin and the pironetin binding site as unexplored targets for cancer therapeutics. Bioorg Med Chem Lett 2019;29:1865–73.

8. Vindya N, Sharma N, Yadav M, Ethiraj K. Tubulins – the target for anticancer therapy. Curr Top Med Chem 2015;15:73–82.

9. Dumontet C, Jordan MA. Microtubule-binding agents: a dynamic field of cancer therapeutics. Nat Rev Drug Discov 2010;9:790–803.

10. Sharma S, Kaur C, Budhiraja A, et al. Chalcone based azacarboline analogues as novel antitubulin agents: design, synthesis, biological evaluation and molecular modelling studies. Eur J Med Chem 2014;85:648–60.

11. Kaur R, Kaur G, Gill RK, Soni R, et al. Recent developments in tubulin polymerization inhibitors: an overview. Eur J Med Chem 2014;87:124.

12. Bukhari SNA, Kumar GB, Revankar HM, Qin HL. Development of combretastatins as potent tubulin polymerization inhibitors. Bioorg Chem 2017;72:130–47.

13. Naumova N, Sachl R. Regulation of cell death by mitochondrial transport systems of calcium and bcl-2 proteins. Membranes 2020;10:299.

14. Thomas E, Gopalakrishnan V, Hegde M, et al. A novel resveratrol based tubulin inhibitor induces mitotic arrest and activates apoptosis in cancer cells. Sci Rep 2016;6:34653.

15. Naaz F, Haider MR, Shafi S, Yar MS. Anti-tubulin agents of natural origin: targeting taxol, vinca, and colchicine binding domains. Eur J Med Chem 2019;171:310–31.

16. Cao YN, Zheng LL, Wang D, et al. Recent advances in microtubule-stabilizing agents. Eur J Med Chem 2018;143:806–28.

17. Cragg GM, Kingston DG, Newman DJ. Anticancer agents from natural products. Boca Raton (FL): CRC press; 2012.

18. Gupta SK, Singh P, Ali V, Verma M. Role of membrane-embedded drug efflux abc transporters in the cancer chemotherapy. Oncol Rev 2020;14:448.

19. Arnst KE, Wang Y, Lei ZN, et al. Colchicine binding site agent d95 overcomes drug resistance and exhibits antitumor efficacy. Mol Pharmacol 2019;96:73–89.

20. Zweifel M, Jayson GC, Reed N, et al. Phase ii trial of combretastatin a4 phosphate, carboplatin, and paclitaxel in patients with platinum-resistant ovarian cancer. Ann Oncol 2011;22:2036–41.

21. Li W, Sun H, Xu S, et al. Tubulin inhibitors targeting the colchicine binding site: a perspective of privileged structures. Future Med Chem 2017;9:1765–94.

22. Kamath PR, Sunil D, Ajees AA. Synthesis of indole-quinoline-oxadiazoles: their anticancer potential and computational tubulin binding studies. Res Chem Intermed 2016;42:5899–914.

23. Khelifi I, Naret T, Renko D, et al. Design, synthesis and anticancer properties of isocombretastatin a-4 phosphate and combretastatin a-4 phosphate with platinum-resistant ovarian cancer. Ann Oncol 2011;22:1025–34.

24. Seligmann J, Twelves C. Tubulin: an example of targeted chemotherapy. Future Med Chem 2013;5:339–52.

25. Piekus-Słomka N, Mikstacka R, Ronowicz J, Sobiak S. Hybrid cis-stilbene molecules: novel anticancer agents. Int J Mol Sci 2019;20:1300.

26. Haider K, Rahaman S, Yar MS, Kamal A. Tubulin inhibitors as novel anticancer agents: an overview on patents (2013–2018). Expert Opin Ther Pat 2019;29:623–41.

27. Das BC, Tang XY, Rogler P, Evans T. Design and synthesis of 3,5-disubstituted boron-containing 1,2,4-oxadiazoles as potential combretastatin a-4 (ca-4) analogs. Tetrahedron Lett 2012;53:3947–50.

28. Biersack B, Effenberg K, Schobert R, Ocker M. Oxazole-bridged combretastatin a-4 analogues with improved anticancer properties. ChemMedChem 2010;5:420–7.

29. Mahal K, Biersack B, Schruefer S, et al. Combretastatin a-4 derived 5-(1-methyl-4-phenyl-imidazol-5-yl)indoles with superior cytotoxic and anti-vascular effects on chemoresistant cancer cells and tumors. Eur J Med Chem 2016;118:9–20.

30. Biernacki K, Daśko M, Ciupaok O, et al. Novel 1,2,4-oxadiazole derivatives in drug discovery. Pharmaceuticals (Basel) 2020;13:111.

31. Schmitt F, Gosch LC, Dittmer A, et al. Oxazole-bridged combretastatin A-4 derivatives with tethered hydroxamic acids: structure(-)activity relations of new inhibitors of hdcac and/or tubulin function. Int J Mol Sci 2019;20:283.

32. Li L, Quan D, Chen J, et al. Design, synthesis, and biological evaluation of 1-substituted-2-aryl imidazoles targeting tubulin polymerization as potential anticancer agents. Eur J Med Chem 2019;184:111732.

33. Ibrahim TS, Hawwas MM, Malebari AM, et al. Potent quinoline-containing combretastatin a-4 analogues: design, synthesis, antiproliferative, and anti-tubulin activity. Pharmaceuticals (Basel) 2020;13:393.

34. Jian YE, Yang F, Jiang CS, et al. Synthesis and biological evaluation of novel pyrazolo[3,4-b]pyridines as cis-restricted combretastatin a-4 analogues. Bioorg Med Chem Lett 2020;30:127025.

35. Yang F, Jian YE, Diao PC, et al. Synthesis and biological evaluation of 3,6-diaryl-[1,2,4]triazolo[4,3-a]pyridine analogues as new potent tubulin polymerization inhibitors. Eur J Med Chem 2020;204:112625.

36. Ma W, Chen P, Huo X, et al. Development of triazolothiadiazine derivatives as highly potent tubulin polymerization inhibitors: structure-activity relationship, in vitro and in vivo study. Eur J Med Chem 2020;208:112847.

37. Kaur K, Jain M, Reddy RP, Jain R. Quinolines and structurally embedded drug efflux abc transporters in the cancer chemotherapy. Oncol Rev 2020;14:448.

38. Li W, Sun H, Xu S, et al. Tubulin inhibitors targeting the colchicine binding site: a perspective of privileged structures. Future Med Chem 2017;9:1765–94.

39. Kamath PR, Sunil D, Ajees AA. Synthesis of indole-quinoline-oxadiazoles: their anticancer potential and computational tubulin binding studies. Res Chem Intermed 2016;42:5899–914.

40. Khelifi I, Naret T, Renko D, et al. Design, synthesis and anticancer properties of isocombretastatin a-4 phosphate and combretastatin a-4 phosphate with platinum-resistant ovarian cancer. Ann Oncol 2011;22:1025–34.

41. Seligmann J, Twelves C. Tubulin: an example of targeted chemotherapy. Future Med Chem 2013;5:339–52.

42. Piekus-Słomka N, Mikstacka R, Ronowicz J, Sobiak S. Hybrid cis-stilbene molecules: novel anticancer agents. Int J Mol Sci 2019;20:1300.
56. Mahapatra DK, Bharti SK, Asati V. Anti-cancer chalcones: structural and molecular target perspectives. Eur J Med Chem 2015;98:69–114.