Trop J Nat Prod Res, August 2023; 7(8):3770-3774 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antitumor Activity of a Quinoline-Substituted Chalcone Epoxide

Muhammad Zahatu[1]*, Hamza, Asmau Nasiru, [1]Ya'u, Jamilu, Magaji Mohammed Garba1, Zezi, Abdulqadir Umar[1]

[1]*Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.*
[2]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences Ahmadu Bello University Zaria, Nigeria*

ARTICLE INFO

Article history:
Received 10 May 2023
Revised 04 August 2023
Accepted 19 August 2023
Published online 01 September 2023

ABSTRACT

Cancer is a multifactorial disease. Chalcones have been identified as potential antitumor agents that target tubulin. This study aims to synthesize and evaluate the antitumor activity of a quinoline-substituted chalcone epoxide as a potential tubulin inhibitor. The compound was synthesized by Claisen-Schmidt condensation and named C1, its structure was established using various spectroscopic techniques. The median lethal dose (LD_{50}) of C1 was evaluated using OECD 425 guidelines, antitumor activity was evaluated in 1-methyl nitrosourea (MNU)-induced mammary tumors in rats. Eight weeks post-MNU administration, the animals were divided into five groups of six rats each and treated with graded doses (12.5, 25, and 50 mg/kg) of C1 and paclitaxel (10 mg/kg) for six weeks. At the end of the treatment, the rats were euthanized, and mammary glands were collected and subjected to histological assessment to confirm tumor induction and assess treatment with C1. The possible mechanism of action of C1 was elucidated *in silico* using molecular docking. The LD_{50} of C1 was above 2000 mg/kg. There was significant decrease (p = 0.041) in mean tumor diameter when compared with the untreated group. Histological assessment shows that the lactiferous gland of the rats treated with MNU and graded doses of C1 showed fewer signs of hyperplasia with a small number of tumor cells in the duct when compared with the cancer control group. Tubulin-binding interaction revealed that C1 and Colchicine have the same binding site. These results suggest that C1 may have potential anticancer activity possibly via microtubule destabilization.

Keywords: Mammary tumor; Chalcone epoxide; Tubulin; Claisen-Schmidt condensation

Introduction

Cancer is one of the major health problems that affect millions of people worldwide.[1,2] There are various strategies employed in treating cancer, including surgery, chemotherapy, and radiation therapy used either alone or in combination. Chemotherapy is still the mainstay in the treatment of cancer. Despite the continuous discovery of new anticancer drugs, their use in cancer treatment is still unsatisfactory due to several adverse effects coupled with resistance and relapse.[3,4] As a result, there is a need to design and develop new anticancer drugs with high selectivity, less toxicity, and better efficacy against all forms of cancers and minimize resistance.[5] Moreover, cancer cells grow and divide very rapidly compared to normal cells. Since the microtubule is one of the critical components required for cell division, microtubule-targeting agents are explored for anticancer drug development.[6]

Microtubules are hollow cylindrical assemblies of tubulin heterodimers involved in several cellular processes such as cell division, where they form the mitotic spindle or intracellular border, and form the paths along which microtubule-based neurons move,[7] making them susceptible targets for anticancer drug design.[8] Currently, numerous microtubule inhibitors (MTIs) such as taxanes, *Vinca* alkaloids, and epothilones are used to manage many solid and hematologic cancers.[9,10]

*Corresponding author. E mail: zahatumuhammad@abu.edu.ng, zahatu2016@gmail.com
Tel: +2348131302991

Citation: Muhammad Z, Hamza AN, Ya'u J, Magaji MG, Zezi AU.. Antitumor Activity of a Quinoline-Substituted Chalcone Epoxide. Trop J Nat Prod Res. 2023; 7(8):37701-3774 http://www.doi.org/10.26538/tjnpr/v7i8.30

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Microtubule inhibitors have been demonstrated to produce a high level of anticancer activity in clinical treatment but are limited by high toxicity and the development of drug resistance.[11] Colchicine was the first identified tubulin destabilizing agent,[12] not approved to treat cancer because of its low therapeutic index and high toxicity, resulting in the development of other colchicine binding site inhibitors (CBSIs). The favorable factors of most CBSIs are that they have little or no multidrug resistance (MDR) issues, simple structure, and are easy to synthesize.[13] Studies revealed that another possible way to prevent cancer multidrug resistance is to develop irreversible binding agents, as hinted by Buey *et al.*, that a small natural molecule such as cyclostreptin retains its activity in cells that are resistant to Paclitaxel by upregulation of P-glycoprotein through covalently binding to β-tubulin.[14] The study suggested that resistant tumor cells cannot elude the effect of compounds irreversibly binding to them by decreasing their affinity for the target or increasing the drug outward. They suggested that designing a compound that irreversibly binds with tubulin could effectively prevent drug resistance. For example, some microtubule-stabilizing agents such as zampanolide and dactyloid retain their activities in MDR cell lines by covalently and irreversibly binding to the taxane binding site in β-tubulin.[15,16] Another tubulin inhibitor that covalently binds to Cys-239 of β-tubulin (T138067) retains full activity in MDR cells both *in vitro* and *in vivo*.[17] The anticancer activity of eribulin on drug-resistant cells was observed to be due to its ability to irreversibly block mitosis and persistently retain drugs in the cancer cells.[18] Several studies evaluated synthesized chalcone's in vitro and in vivo toxicity and their epoxides were reported to be relatively non-toxic. [19,20]

These findings showed that irreversible modification of tubulin will destroy cancer cells and may also overcome MDR. This study is aimed at synthesizing a new anticancer agent, from the chalcone family; quinolinyl-chalcone epi-oxide (compound 1) of less toxicity and probably more ability to counter drug resistance.

Materials and Methods

Materials

Equipment: Melting points are uncorrected and, were determined in a Gallenklamp melting point apparatus. Fourier Transform Infrared (FT-IR) spectrum was performed on an Agilent model 470 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a 400 MHz Bruker spectrometer and are reported in ppm downfield from TMS as an internal standard.

Reagents, solvents, and standard drugs; All the starting reagents and solvents used for the experiments were of analytical grade and were used without further purification, these include 2-chloro-6-methoxy-3-quinoline carbaldehyde, 2, 4-dimethoxy acetophenone, sodium hydroxide (50%), iodine crystals, chloroform, ethyl acetate, hexane, ethanol, hydrochloric acid, 10% Giemsa stain, Acacia, 1-methyl nitroso-urea, Paclitaxel. The reagents were purchased from Sigma Aldrich Germany.

Synthesis of compound 1; Compound 1 was synthesized via a base-catalyzed condensation of 2, 4-dimethoxy-acetophenone (0.01 mole), and 2-chloro-6-methoxy-3-quinoline carbaldehyde (0.01 mole) with 20 ml ethanol in a round bottom flask. To this 10ml of 40%, sodium hydroxide solution was added dropwise with continuous stirring for 30 minutes at 25^{0}C. The mixing was then continued for another 12 hours at room temperature using a magnetic stirrer. The resulting mixture was kept in a refrigerator overnight until it formed a colored solid mass. Completion of the reaction was established by the disappearance of spots due to reactants on thin-layer chromatograms and the appearance of a single spot.

Drops of 10% HCl were added to neutralize the reaction medium. The mixture was further diluted with 40 ml of ice-cold distilled water and then filtered; the residue was washed with more ice-cold distilled water and dried in air. The product was recrystallized with ethanol, dried, and weighed.

Characterization of compound 1; Structural characterization of the compound was performed using FT-IR and, ^{1}H, ^{13}C, and 2D-NMR Spectroscopy. FT-IR data are reported in terms of frequency of absorption ν cm-^{1}. Data for ^{1}H-NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, dd = doublet of a doublet, m = multiplet), integration (J in Hz). Data for ^{13}CNMR expressed as chemical shift δ (ppm).

In-Vivo Antitumor Study

Animal management

Ethical approval was sought from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), with the approval number ABUCAUC/2022/003. Adult female Wistar rats weighing between 60-80 g were obtained and housed in the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The rats were fed with standard feed and allowed water *ad libitum.*

Acute toxicity studies in rats

The oral median lethal dose (LD_{50}) was determined using OECD 425[21] guidelines in rats. Three rats were fasted before treatment for 3 hours, fasted body weight was determined for each animal, and the dose was calculated according to the body weight. Food was further withheld for two hours after the administration of compound 1. A rat was dosed at 2000 mg/kg (limit test) and was observed for 48 hours. The first rat survived; thus, additional two rats were dosed with 2000 mg/kg. The rats were observed for signs and symptoms of toxicity at least once during the first 30 minutes, periodically during the first 24 hours, and then daily for 14 days after dosing.

Induction of mammary tumor

Female Wistar rats at P45-50 were used for the study. Before tumor induction, the rats were immunosuppressed with a single dose of Dexamethasone (10 mg/kg). The mammary tumor was induced according to the method of Thompson *et al.*[22] with modification. Each rat was administered 65 mg/kg of 1-methyl nitrosourea dissolved in normal saline subcutaneously, beneath the mammary gland. The rats were observed and palpated weekly to determine the development, localization, and size of neoplasia on the mammary gland. Eight weeks post-cancer induction, the rats were divided into five groups of six rats each. Rats in group I served as negative control and received normal saline (1 ml/kg), group II, III, and IV rats were treated with graded doses of compound 1 (50, 25, and 12.5 mg/kg respectively via oral route) daily, while rats in group V served as positive control and received Paclitaxel (10 mg/kg, *i.p*) on alternate days. The treatment lasted six weeks.

Sample collection

At the end of the study, rats were sacrificed, and mammary tissue samples were collected, weighed, and preserved in 10%$^{v}/_{v}$ formalin in normal saline for histological assessment.[23]

Histological examination

The specimens from each rat were immediately stored in 10%$^{v}/_{v}$ formalin in normal saline after gross histological examination and dehydrated using increasing concentrations of isopropyl alcohol. Paraffin sections at 5 mm thickness were made from the paraffin-embedded organs using a Leica rotary microtome. This was followed by routine staining with hematoxylin and eosin, which involved the process of deparaffinization, hydration, staining, rinsing, and clearing in xylene. Slides were viewed under a light microscope with photomicrographs taken with a Leica DM750 Camera Microscope (X 250).[23]

Molecular Docking

Target preparation

The enzyme tubulin was obtained from a protein data bank with PDB ID 4O2B. Best-resolved monomers were chosen for the study. All non-standard residues were removed from the 3D structures of the enzymes using UCSF Chimera. Isolated receptor and co-crystallized ligand (colchicine) were prepared on Chimera and saved as a rec. pdb and Lig.mol2, respectively. Rec.pdb and Lig.mol2 were edited on the Autodock tool (ADT) by adding polar hydrogen and Gastier charges then saved as pdbqt files.

Ligand structure

The 2D structure of the ligand (compound 1) was generated using Chem Draw. 3D structure generation and geometric optimization using the AMI semi-empirical method were performed with Spartan 06. Polar hydrogen and Gasteir charges were added and converted to pdbqt using ADT.

Docking was carried out using the virtual screening software Auto Dock Vina.[24] The ligand was docked on the active site of the protein. The docking procedure for the protein was validated before docking the test compound. The best pose was selected from the View Dock feature of Chimera and saved in a complex with the reference protein.

Data analysis

Statistical analysis was carried out using SPSS (Version 20) and data obtained were expressed as mean ± SEM. Differences between mean were analyzed using one-way or repeated measures analysis of variance (ANOVA) where applicable, then, followed by Bonferroni post hoc test, for multiple comparisons, values with $p \leq 0.05$ were considered significant.

Results and Discussion

The two-dimensional (2D) representation of compound 1 is showed in (Figure 1). The synthesis of the compound furnishes good yields of 78.04%, some of the physicochemical properties of the compound are shown in table (Table 1). The orange color and the crystalline nature of the compound agree with the appearance of natural chalcones and other chalcone derivatives reported in the literature.[25,26] The sharp melting point observed with the compound suggests that the compound has a high degree of purity[27].

From the FT-IR data (supplementary F1), the olefinic frequency observed in the region 2995-3008 cm^{-1} is evident that the chalcone has been formed. Also, the characteristic α, the β-unsaturated carbonyl group of the chalcones was confirmed by the prominent band between

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

1625-1650 cm-[1]. Additionally, the methoxy function is evidenced by coupled strong vibration frequency at 1260 and 1095 cm^{-1} for C-O-C stretching vibrations.[28]

The ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra were used to elucidate the structure of compound 1. A noticeable change from the starting materials is the disappearance of the aldehyde and methyl ketone chemical shifts, which appear as singlets around 9.89-10.30 ppm and 2.50 ppm respectively. These protons are replaced by the oxy-methyl protons of an epi-oxide observed as doublets in the region of 4.79 – 4.78 ppm (H- α) and 4.92 – 4.94 ppm (H- β) with coupling constants of 6.4 Hz. The other noticeable observations in the H NMR are the methoxy proton as singlets at the region of 3.83 – 4.25 ppm. The rest of the protons appear in their expected regions with their usual coupling constants (Supplementary information).

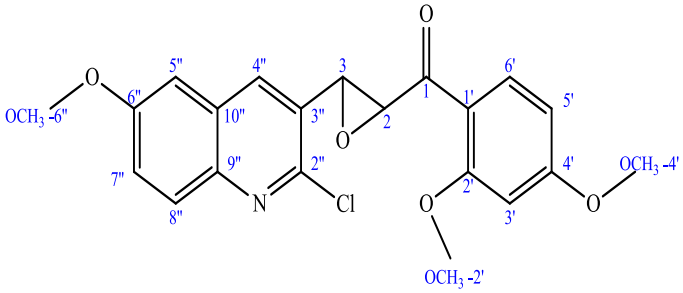

Figure 1. Quinolinyl-chalcone epi-oxide, (3-(2-chloro-6-methoxyquinolin-3-yl) oxirane-2-yl)

Table 1. Yield and some physical properties of compound 1

Compound's ID	Color	R_f Values	Melting points (^0C)	% Yield
Compound 1	Orange crystals	0.40	120-123	50.64

(a) Silica, Thin layer chromatography plate was used with n-hexane: ethyl acetate (7:3) as development solvent. R_f is a retention factor.
Characterization of Compound 1

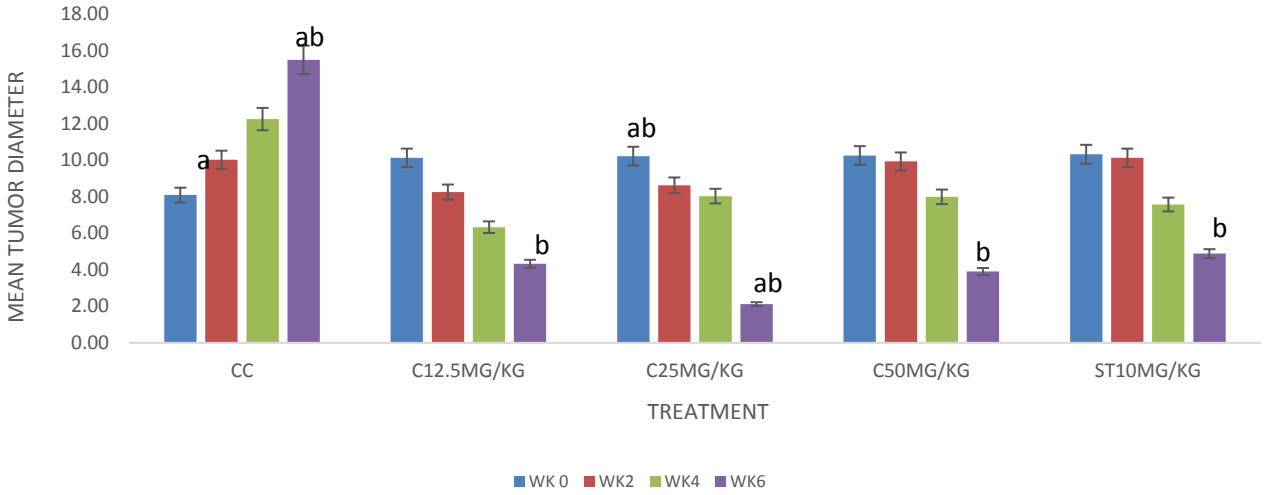

Figure 2: Effects of compound 1 administration on average tumor diameter in MNU-induced mammary tumor rats
Values are expressed as Mean ± S.E.M. Data was analyzed using repeated measures ANOVA followed by Bonferroni post hoc test. n=6, C = Compound 1, CC =cancer control, ST = Paclitaxel a = difference within, b = difference between the groups

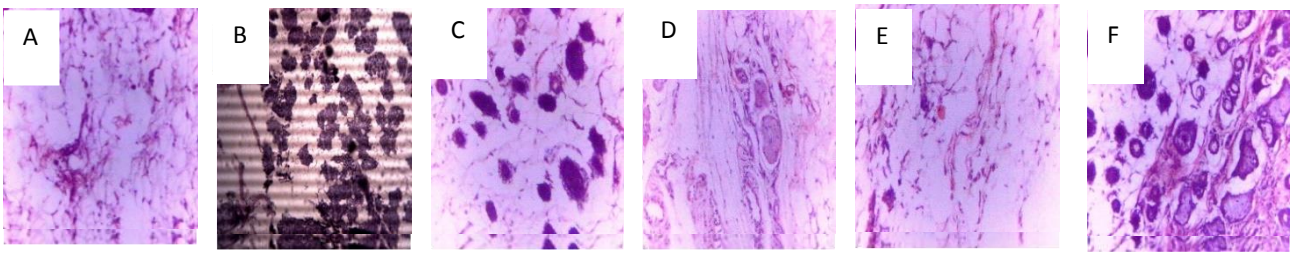

Plate I. Photomicrograph of a section of the mammary gland of female Wistar rats showing the lactiferous glands of the rats treated with normal saline, MNU, and compound 1.

A=Normal saline showed a normal feature of the lactiferous gland
B=1-Methyl nitrosourea and normal saline showing dilated ducts filled with tumor cells and extreme hyperplasia of mammary lobules and decreased connective tissue of the lactiferous gland (H and E X 250).
C= 1-Methyl nitrosourea and Paclitaxel (10 mg/kg) showing dilated ducts filled with tumor cells and moderate hyperplasia of mammary lobules and slightly decreased connective tissue (H and E X 250)
D= 1-Methyl nitrosourea and compound 1 (50 mg/kg) displayed fewer signs of hyperplasia and small numbers of connective tissue with larger lobules (H and E X 250).
E= 1-Methyl nitrosourea and compound 1 (25 mg/kg) displayed fewer signs of hyperplasia and small numbers of connective tissue with larger lobules (H and E X 250).
F= 1-Methyl nitrosourea and compound 1 (12.5 mg/kg) showing dilated ducts filled with tumor cells and moderate hyperplasia of mammary lobules and slightly decreased connective tissue (H and E X 250)

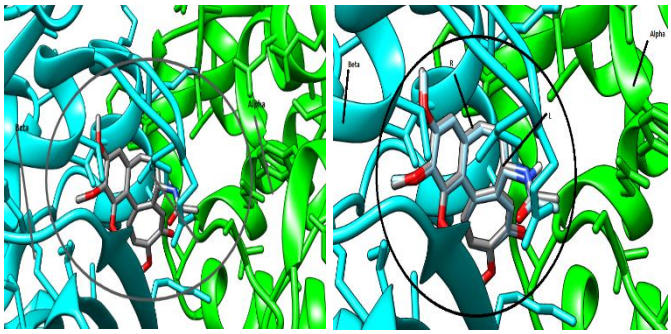

Figure 3: Molecular docking validation with the co-crystallized ligand (colchicine) of Tubulin protein (PDB ID 4o2b)

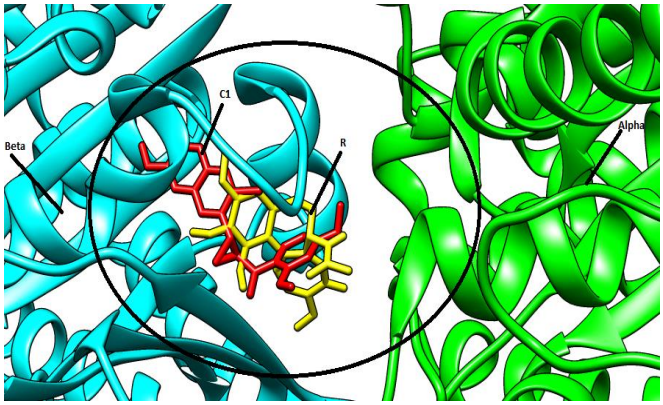

Figure 4: Lid ribbon representation of 4O2B docked with the crystal structure of compound 1 (red) and the Colchicine (yellow) in the active site

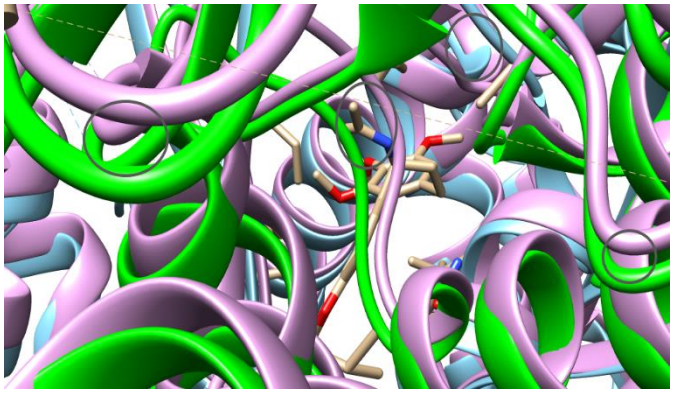

Figure 5: Lid ribbon representation of 4O2B (green/ cyan) docked with the crystal structure of compound 1 (colored by elements) in the active site and the apo-tubulin (magenta). The black circles indicate the structural difference between the two proteins.

Additional support for the structures of the compound comes from the ^{13}C NMR spectra, where the carbonyl carbon (C=O) of the *α, β*-unsaturated ketone linker is observed in the region 191 ppm, compared to the aldehyde carbonyl group (C=O) at 195 ppm and methyl ketone carbonyl group (C=O) which appears in the region 179 ppm. The *α* and *β*- carbon atoms concerning the carbonyl group give rise to characteristic signals in being δ 53.1 ppm and δ 41.4 ppm respectively. The methyl carbons (α- and β- carbon to the carbonyl carbon) are evidence that a chalcone epi-oxide has been formed. The positions of all C atoms in the compound have been assigned.[29]

In the *in vivo* study, an acute toxicity study revealed, that, C1 is relatively safe with LD_{50} above 2000mg/kg and also displayed a significant antitumor activity, having activity better than that of Paclitaxel (10 mg kg^{-1}) at a dose of 50 and 25 mg kg^{-1}. The antitumor activity of the compound was assessed by measuring tumor diameter which showed a significant decrease (p = 0.041) in the mean weekly tumor diameter when compared with the untreated group (Figure 2). Histological evaluation was deployed to corroborate the antitumor activity of the C1. Induction of cancer is characterized by a dilated duct that is filled with tumor cells, a small number of connective tissues, and extreme hyperplasia of lobules. All the aforementioned features were seen in the group of rats treated with MNU and normal saline (cancer control group). The lactiferous gland of the groups of rats treated with MNU and graded doses of C1 showed fewer signs of hyperplasia with a small number of tumor cells in the duct when compared with the cancer control group (Plate 1). The ability of the compound to reduce the tumor cells and hyperplasia, support its antitumor activity. This finding is in line with the work of Zingales and Moore who reported that synthesized chalcones and their derivatives contained potential leading skeletons of antiproliferative agents, and modifications in their skeletal structure enhanced their antitumor activities.[30] Lawrence and colleagues also reported the synthesis of a 644-membered library of chalcones by parallel synthesis using the Claisen-Schmidt reaction.[31] Seven chalcones exhibited an IC_{50} of less than 1 µM against K562 cells. The most active compound was impressively cytotoxic and inhibited tubulin polymerization.[32] Ducki and colleagues also incorporated the aryl substitution into chalcones and obtained several chalcones with substantial *in vitro* activity against the K562 human leukemia cell line.[33] As shown in the SAR analysis, the 3,4,5-tri methoxy A-ring substitution distinctly increases cytotoxicity. The most potent compound of a series of novel dithiocarbamate compounds with the chalcone scaffold inhibited the growth of MCF-7 cells tubulin polymerization similar to Colchicine.[34] In addition to individual compounds, several potent hybrid molecules with anticancer activities have been developed by grouping different pharmacophores. For example, the hybrid of imidazothiazoles and chalcones resulted in 2,3-diaryl imidazo [1,2-b] thiazole-chalcone conjugates that exhibited significant antiproliferative activities against several cancer cells.[35,36]

On the determination of the possible mechanism of action of compound 1 using molecular docking to determine the tubulin binding interactions. The docking procedure was well validated and the cocrystal ligand (colchicine) is well superimposed with the docked ligand (Figure 3). Tubulin-binding assays revealed that the kinetics of compound 1 binding to tubulin were similar to that of Colchicine, with evidence of competition between compound 1 and Colchicine for the binding site. A comparison of crystal structures of tubulin-compound 1 and tubulin–colchicine complexes show that the binding mode of compound 1 to tubulin is similar to that of Colchicine (Figure 4). When colchicine binds to its site, it blocks a helix which is at the interface with tubulin from stacking onto a tubulin sheet as in straight protofilaments (Figure 5). While in the presence of these ligands, the interference with microtubule assembly gets frozen, by flipping in and out the subunit, a loop participates reversibly in the resistance to straightening that opposes microtubule assembly.[36] The antitumor activity of compound 1 seen in the MNU-induced mammary tumor rats could be a result of the ability of compound 1 to inhibit tubulin polymerization by binding to the colchicine binding site. Our results suggest that compound 1 contributes to microtubule dynamic instability. Taken together, our data suggest that compound 1 could be a potent tubulin inhibitor with distinct effects on microtubule organization.

Conclusion

This study showed that a quinolinyl-chalcone epi-oxide was synthesized with a very good yield, was relatively safe, and possessed anticancer activity. Molecular docking studies revealed that the compound could bind to the colchicine site of tubulin and inhibit its activity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. National Comprehensive Cancer Network (NCCN). Practice guidelines in oncology: breast cancer. Version 3. [online] 2014 Available from: www.nccn.org.
2. World Health Organization. Cancer facts sheet. No 307" 2019.
3. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistic. Cancer J. 2005; 53:74-108.
4. Fasoranti TO. Combating breast cancer in Nigeria; the need for comprehensive screening programs. [online] 2012 (cited 2011) available from: http://www.gamji.com/article600/News7489htm.
5. Nepali K, Sharma S, Sharma M, Bedi PMS, Dhar KL. Rational approaches, design strategies, structure-activity relationship and mechanistic insights for anticancer hybrids, Eur J Med Chem. 2014; 77: 422-487.
6. Petrelli A, Giordano S. From single- to multi-target drugs in cancer therapy: When a specificity becomes an advantage. Curr Med Chem. 2008; 15: 422-432.
7. Perez EA. Microtubule inhibitors: Differentiating tubulin-inhibiting agents based on mechanisms of action, clinical activity, and resistance. Mol Cancer Therapeutics 2009; 8: 2086.
8. Jordan MA, Kamath K. How do microtubule-targeted drugs work? An overview. Curr Cancer Drug Targets 2007; 7: 730.
9. Bhalla KN. Microtubule-targeted anticancer agents and apoptosis. Oncogene 2003; 22: 9075.
10. Kim JS, Lee YC, Nam HT, Li G, Yun EJ, Song KS, Seo KS, Park JH, Ahn JW, Zee O. Apicularen A induces cell death through fas ligand up-regulation and microtubule disruption by tubulin down-regulation in hm7 human colon cancer cells. Clin. Cancer Res. 2007; 13: 6509-6517.
11. Kavallaris M. Microtubules and resistance to tubulin-binding agents. Nat Rev Cancer 2010; 10: 194.
12. Lockwood AH. Molecules in the mammalian brain that interact with the colchicine site on tubulin. Proc Natl Acad Sci U S A. 1997; 76: 1184.
13. Lu Y, Chen J, Xiao M, Li W, Miller DD. An overview of tubulin inhibitors that interact with the colchicine binding site. Pharm. Res. 2012; 29: 2943-2971.
14. Buey RM, Calvo E, Barasoain I, Pineda O, Edler MC, Matesanz R, Cerezo G, Vanderwal CD, Day BW, Sorensen EJ. Cyclostreptin binds covalently to microtubule pores and luminal taxoid binding sites. Nat Chem Biol. 2007; 3: 117.
15. Field JJ, Pera B, Calvo E, Canales A, Zurwerra D, Trigili C, Rodríguezsalarichs J, Matesanz R, Kanakkanthara A, Wakefield SJ. Zampanolide, a potent new microtubule stabilizing agent, covalently reacts with the taxane luminal site in both tubulin α, β-heterodimers, and microtubules. Chem Biol 2012; 19: 686.
16. Prota AE, Bargsten K, Zurwerra D, Field JJ, Díaz JF, Altmann KH, Steinmetz MO. Molecular mechanism of action of microtubule-stabilizing anticancer agents. Science 2013; 339**:** 587.
17. Shan B, Medina JC, Santha E, Frankmoelle WP, Chou T, Learned RM, Narbut MR, Stott D, Wu P, Jaen JC. Selective, covalent modification of β-tubulin residue cys-239 by t138067, an antitumor agent with in vivo efficacy against multidrug-resistant tumors. Proc Natl Acad Sci U S A 1999; 96: 5686-5691.
18. Sanat KMS. Rational drug design. Eur J Pharmacology. 2009; 90-100.
19. Lu Y, Chen J, Xiao M, Li W, Miller DD. An overview of tubulin inhibitors that interact with the colchicine binding site. Pharm. Res. 2012; 29: 2943-2971.
20. Cancino K, Castro I, Yaur C, Jullian V, Arévalo J, Sauvain M, Adaui V, Castillo D. Toxicity assessment of synthetic chalcones with antileishmanial potential in balb/C mice. Rev. Peru. Med. 2021; 38: 424-433.
21. Organization for Economic Cooperation and Development. OECD Guidelines for testing of chemicals, acute oral toxicity. 2001; 423: 1-14
22. Kluver H, Barrera E. A method for the combined staining of cells and fibers in the nervous system. J. Neuropath Exp. Neurol. 1953; 12:400-410
23. Thompson HJ, Adlakha H. Dose-responsive induction of mammary gland carcinomas by the intraperitoneal injection of 1-methyl-1-nitrosourea. Cancer Res. 1992; 51:3411–3415.
24. Towle MJ, Salvato KA, Wels BF, Aalfs KK, Zheng W, Seletsky BM, Zhu X, Lewis B. Eribulin Induces irreversible mitotic blockade: implications of cell-based pharmacodynamics for in vivo efficacy under intermittent dosing conditions. Cancer Res 2011; 71: 496-505.
25. Bandgar BP, Gawande SS, Bodade RG, Totre JV, Khobragade CN. Synthesis and biological evaluation of simple methoxylated chalcones as anticancer, anti-inflammatory, and antioxidant agents. Bioorganic Med Chem. 2010; 18: 1364-1370.
26. Lipinski CA. Lead-and drug-like compounds: the rule-of-five revolution. Drug discovery today. Technologies. 2004; 4: 337-341.
27. Kumar B, Singh S, Skvortsova I, Kumar V. Promising targets in anticancer drug development: Recent updates. Curr med chem. 2007; 24: 4729-4752.
28. Gomes MN, Muratov EN, Pereira M, Peixoto JC, Rosseto LP, Cravo PVL, Neves BJ. Chalcone derivatives: Promising starting points for drug design. molecules. 2017; 22. doi:Doi:https://doi.org/10.3390/molecules22081210.
29. Zingales SK, Moore ME. Design and synthesis of (2-(furanyl)vinyl)-1-tetralone chalcones as anticancer agents. Der Pharma Chemica. 2016; 8: 40–47.
30. Lawrence T, Gilroy DW. Chronic inflammation: a failure of resolution? Int J Exp Pathology. 2007; 88: 85-94. doi:DOI: 10.1111/j.1365-2613.2006.00507.
31. Dong M, Liu F, Zhou H, Zhai S, Yan B. Novel natural product-and privileged scaffold-based tubulin inhibitors targeting the colchicine binding site. Molecules. 2016; 21. doi https://doi.org/10.3390/molecules21101375.
32. Ducki S. The development of chalcones as promising anticancer agents. JDID. 2007; 10: 42–46
33. Shan B, Medina JC, Santha E, Frankmoelle WP, Chou T, Learned RM, Narbut MR, Stott D, Wu P, Jaen JC. Selective, Covalent modification of β-tubulin residue Cys-239 by T138067, an antitumor agent with *in vivo* efficacy against multidrug-resistant tumors. Proc Natl Acad Sci U S A. 1999; 96: 5686-5691.
34. Prota AE. Molecular mechanism of action of microtubule-stabilizing anticancer agents. Science. 2013; 3: 587.
35. Prota AE, Danel F, Bachmann F, Bargsten K, Buey RM, Pohlmann J, Steinmetz MO. The novel microtubule-destabilizing drug bal27862 binds to the colchicine site of tubulin with distinct effects on microtubule organization. Mol Biol. 2014; 462: 1848–1860. Doi https://doi.org/10.1016/j.jmb.2014.02.005.
36. Banerjee S, Arnst KE, Wang Y, Kumar G, Deng S, Yang L. Heterocyclic-fused pyrimidines as novel tubulin polymerization inhibitors targeting the colchicine binding site: structural basis and antitumor efficacy. Med Chem. 2018; doi: https://doi.org/10.1021/acs.jmedchem. 7b01858.