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Research Article

Experimental and Molecular Docking Study of 3′,4′,5′-Trimethoxychalcones Targeting Overexpressed Protein in HCT-116 Colon Cancer Cells

Ade Danova[1*], Piyanuch Wonganan[2], Elvira Hermawati[1], Fera Kurniadewi[3], Iqbal Musthapa[4], Warinthorn Chavasiri[5*]

[1]*Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung, Jl. Ganesha 10, Bandung, West Java 40132, Indonesia*
[2]*Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand*
[3]*Chemistry Study Program, Universitas Negeri Jakarta, Jalan Rawamangun Muka, Jakarta 13220, Indonesia*
[4]*Chemistry Study Program, Faculty of Mathematics and Natural Science Education, Universitas Pendidikan Indonesia, Jl. Dr. Setiabudi No 229, Bandung 40154, Indonesia*
[5]*Center of Excellence in Natural Products Chemistry, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*

[*]*Email: adedanova@itb.ac.id; warinthorn.c@chula.ac.th*

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Abstract

Cancer poses a substantial global health challenge. Colorectal cancer (CRC) is the second leading cause of cancer-related mortality after lung cancer and is associated with high mortality rates worldwide. Chalcones have attracted significant interest because of their diverse biological properties, including potential anticancer effects. In this study, five 3′,4′,5′-trimethoxychalcones (**1**-**5**) were tested against HCT-116 colon cancer cells using an MTT assay for the first time. Molecular docking was conducted to predict molecular interactions targeting three proteins (tubulin, EGFR, and CDK2). Among the five, four compounds (**1**, **3**, **4**, and **5**) exhibited strong inhibitory activity against HCT-116 colon cells, with IC_{50} values < 10 µM. Compounds **1**-**5** showed potency as drug candidates based on the Lipinski rules and pharmacokinetic profiles using SwissADME and pkCSM online tools. Moreover, molecular docking was performed on compound **5** against three protein targets (tubulin, EGFR, CDK2) with binding affinities of -7.4, -7.3, and -8.5 kcal/mol, respectively, and showed major H-bond interactions. Therefore, these results suggest that compound **5** could be a potential inhibitor to be developed in future studies, both *in vitro* and *in vivo*, to understand its inhibition mechanism and efficacy.

Keywords: Colon cancer, chalcone, HCT-116, inhibitor, molecular docking

1. INTRODUCTION

Cancer is a significant public health concern, ranking as the second most prevalent cause of mortality following cardiovascular diseases. According to GLOBOCAN 2020 estimates produced by the International Agency for Research on Cancer, there were approximately 19.3 million new cancer cases and nearly 10.0 million cancer-related deaths globally in 2020. Colorectal cancer (CRC) is the second leading cause of cancer-related mortality after lung cancer and is associated with high mortality rates worldwide [1].

Chalcones, a subset of polyphenolic compounds within the extensive flavonoid family, are widely distributed across the plant kingdom and are found in various fruits and vegetables [2,3]. These compounds are distinguished by two aromatic rings connected with a three-carbon α,β-unsaturated ketone [4]. Chalcones have attracted significant attention because of their diverse biological activities, including antioxidant [5], anti-inflammatory [6], antibacterial [7], antiviral [8], antimalarial [9], antityrosinase [10], and anticancer properties [11,12]. In terms of structure–activity relationships, the presence of a trimethoxyphenyl ring has been identified as crucial for the anticancer efficacy of chalcones [13-20]. Moreover, 3′,4′,5′-trimethoxychalcones have been reported in several cancer cell lines, such as human lung (A549 and H2009), K562 (human leukemia),

human breast (MCF-7 and BT20), A2780 (human ovary), A2780/ADR (human ovary, multidrug resistant), HepG2 (human hepatoma), colon 205 (human colon), L1210 (murine leukemia), FM3A (murine mammary carcinoma), human T-lymphocyte (Molt/4 and CEM), HeLa (human cervix carcinoma), human colorectal (HCT-116 and HT-29), and human prostate (PC-3 and DU-145) [15,16,21-26].

Microtubules, which are crucial components of the cytoskeleton in eukaryotic cells, play an important role in various cellular functions, including maintaining cell shape, transmitting signals, regulating organelles, enabling cell movement, division, and mitosis [27]. The epidermal growth factor receptor (EGFR) is a cell surface receptor with tyrosine kinase activity that is highly expressed. When this transmembrane receptor is activated through phosphorylation, it promotes the proliferation of cancer cells, thereby inhibiting apoptosis and facilitating metastasis and invasion [28]. Additionally, Cyclin-dependent kinase 2 (CDK2) is identified as a key player in cell cycle regulation and is considered a promising target for cancer treatments, especially in the context of colorectal cancer (CRC) [29]. Recently, many trimethoxyphenyl moieties attached to the structures can inhibit tubulin polymerization, protein kinases (e.g, EGFR), and cell cycle processes [16,19,30,31], as presented in **Figure 1**. Therefore, this study aimed to evaluate the antiproliferative effects of five 3′,4′,5′-trimethoxychalcones, as presented in **Figure 2**. To the best of our knowledge, these compounds have not been reported against HCT-116 colon cancer cells. Furthermore, molecular docking was performed to predict the molecular interaction between ligand and amino acid residues in the three protein targets, which were vital to inhibit the growth of cancer cell lines, such as tubulin, EGFR, and CDK2.

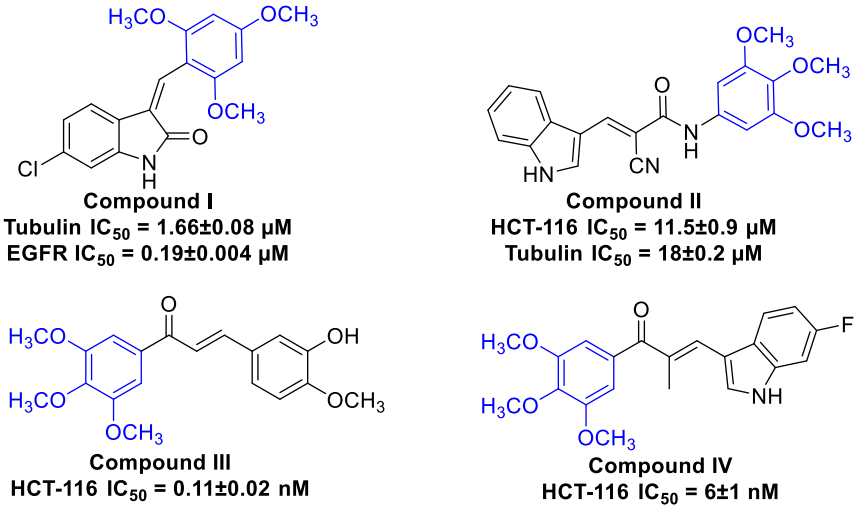


Figure 1. Compounds possessing trimethoxyphenyl (blue color) as potent anticancer (**I**-**IV**).

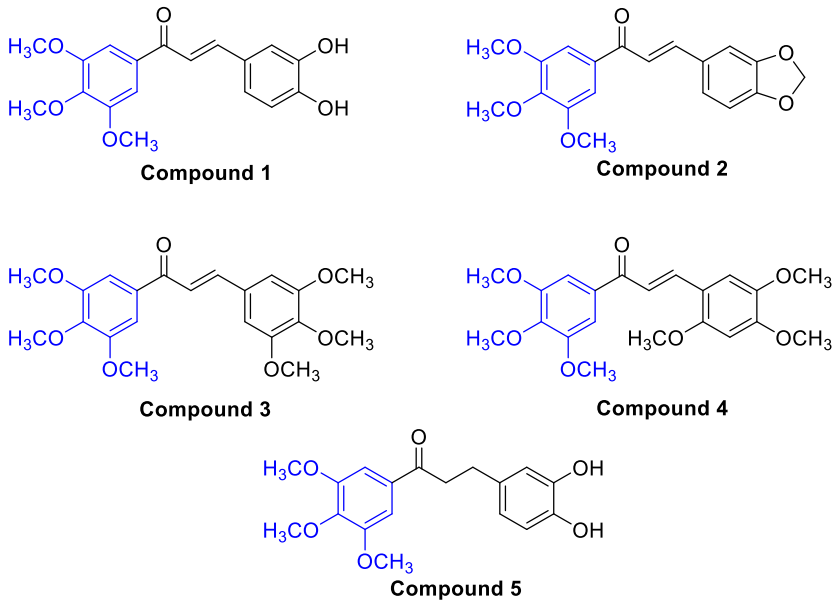


Figure 2. Rational Design of Five 3′,4′,5′-trimethoxychalcones (**1**-**5**) against HCT-116 colon cancer cells.

2. RESEARCH METHODS

Instruments and Materials

The human colon carcinoma cell line HCT-116 was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Five 3′,4′,5′-trimethoxychalcones were obtained from our previous work [15]. Molecular docking was performed using PyRx V.1.1 software. The IUPAC names of the compounds in this study were as follows:

1) Compound **1**: (*E*)-3-(3,4-dihydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one
2) Compound **2**: (*E*)-3-(benzo[d][1,3]dioxol-5-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one
3) Compound **3**: (*E*)-1,3-bis(3,4,5-trimethoxyphenyl)prop-2-en-1-one
4) Compound **4**: (*E*)-3-(2,4,5-trimethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one
5) Compound **5**: 3-(3,4-dihydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)propan-1-one

Cytotoxic Assay

A cytotoxicity assay was conducted as previously described [32]. To identify formazan products, HCT-116 colon cancer cells were seeded at a density of 5×10^4 cells/mL in 96-well plates and incubated overnight. Subsequently, the cells were treated with chalcones or oxaliplatin (utilized as a positive control) at varying concentrations, or with 0.2% DMSO (serving as a vehicle control) in a complete medium for 48 hours. Following this, an MTT solution (0.5 mg/mL) was added and incubated for an additional 4 hours. The medium was then removed, and 150 µL of 0.2% DMSO was added to dissolve the formazan crystals. Finally, the absorbance of the formazan product was measured at 570 nm using a microplate reader from Thermo Fisher Scientific, Vantaa, Finland.

Drug-likeness and pharmacokinetic properties

The properties of drug-likeness and pharmacokinetic prediction of five compounds (**1**-**5**) were determined using SwissADME (https://swissadme.ch/) and pkCSM (https://biosig.lab.uq.edu.au/pkcsm/) web servers [33,34].

Molecular Docking Study

The molecular structures of the compounds were drawn and refined using the molecular mechanics energy minimization method with the Merck Molecular Force Field (MMFF94) in ChemOffice Professional 15.0. Crystal structures of the three protein targets, tubulin (PDB ID: 5LYJ), EGFR (PDB ID: 1M17), and CDK2 (PDB ID: 6GUE), were retrieved from the Protein Data Bank (https://www.rcsb.org/). All proteins were prepared using BIOVIA Discovery Studio Visualizer to remove all ligand and water molecules and then submitted it to PyRx AutoDock to make macromolecules. Molecular docking was conducted using the AutoDock Vina tool, compiled in PyRx V. 1.1 [35,36], with an exhaustiveness of 32 and a mode value of nine poses for each docked ligand. The redocking process was performed to calculate the location of the binding pocket based on the native ligand. The coordinates of binding pocket for three protein targets with dimensions 40 x 40 x 40 Å, such as tubulin (X: 18.507, Y: 66.627, and Z: 42.148 Å), EGFR (X: 22.220, Y: -1.840 and Z: 55.151 Å), CDK2 (X: -8.528, Y: -22.224, and Z: 23.704 Å). All redocking processes were validated by determining the RMSD value ≤ 2.0 Å [37]. The final step was performed by binding interaction analysis and visualizing the docking results in 3D using BIOVIA Discovery Studio Visualizer.

3. RESULTS AND DISCUSSION

Five 3′,4′,5′-Trimethoxychalcones were synthesized as previously described [15]. This study evaluated five compounds (**1**-**5**) that had not been previously reported against human colorectal cancer cell lines (HCT-116) using an MTT assay (**Figure 2**). Oxaliplatin, used as a positive control, exhibited anticancer activity against HCT-116 colon cancer cells with an IC_{50} value of 4.61±0.22 µM based on the previous report [32]. Among the five compounds, four (**1**, **3**, **4**, and **5**) exhibited strong activity against HCT-116 colon cells, with IC_{50} values < 10 µM, except for compound **2** (**Figure 3**). These results revealed that compounds **1**, **3**, **4,** and **5**, which possess dihydroxy and trimethoxy groups on the B-ring, showed strong inhibitory activity against HCT-116 colon cancer cells. Furthermore, five compounds (**1**-**5**) were determined the drug-likeness and ADMET profiles using SwissADME and pkCSM online tools [33,34]. As presented in **Tables 1** and **2**, five compounds (**1**-**5**) were suitable for Lipinski's rules without toxicity and low permeability to the blood-brain barrier. However, compounds **1** and **2** showed very low total clearance compared with others.

In addition, compound **1** exhibited strong inhibitory activity against HCT-116 colon cancer cells (Figure 3), but this compound was toxic to the normal human primary dermal fibroblasts (PCS201-010) with IC_{50} of 0.86±0.04 µM, as our previous report [15]. Compound **5**, derived from **1** via olefin hydrogenation, exhibited strong activity against HCT-116 colon cancer cells (**Figure 3**) but weak anticancer activity against A549 cells [15]. Moreover, compound **5** displayed anti-fibrotic potency in chronic kidney disease without cytotoxic activity against normal human renal proximal tubule epithelial cells (RPTEC) in our previous study [38]. Therefore, compound **5** is a potential candidate for further study as an anticancer agent against HCT-116 colon cancer cells.

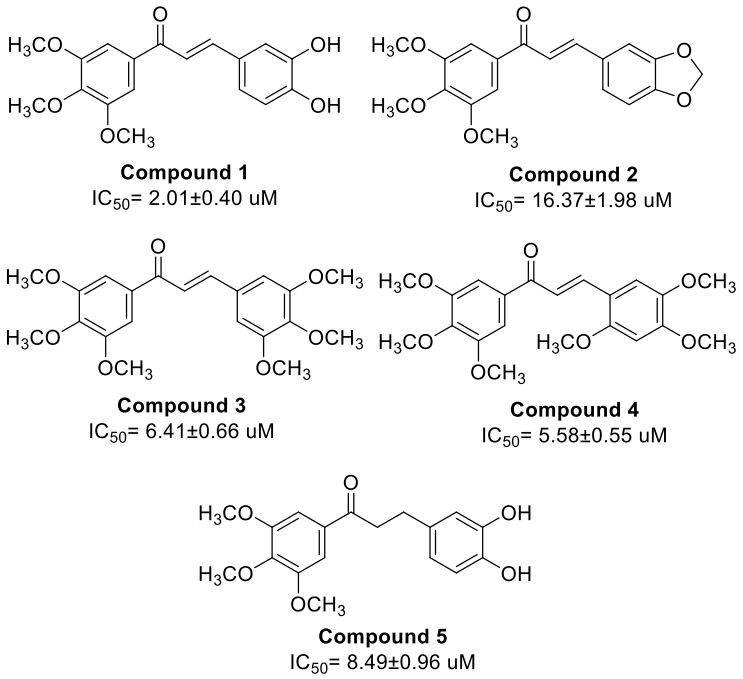


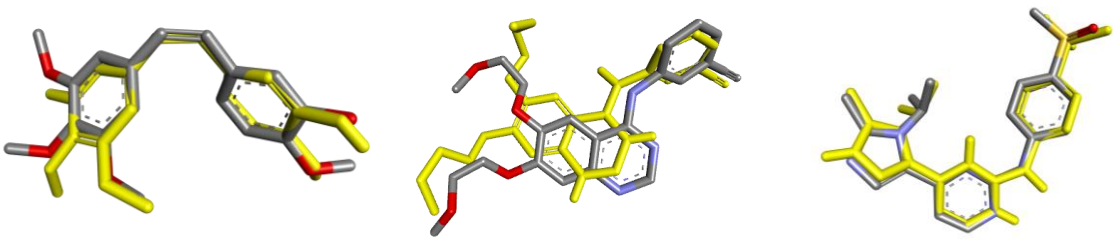
Figure 3. IC_{50} values of five 3′,4′,5′-trimethoxychalcones (**1**-**5**) against HCT-116 colon cancer cells

Table 1. Drug-likeness of five compounds (**1**-**5**) using SwissADME

Compound	MW (≤ 500 Da)	TPSA (≤ 140 Å^2)	Log P (≤ 5)	Rotatable Bonds (≤ 10)	HBA (≤ 10)	HBD (≤ 5)	Bioavailability Score	Lipinski Violations
1	330.33	85.22	2.56	6	6	2	0.55	No
2	342.34	63.22	3.12	6	6	0	0.55	No
3	388.41	72.45	3.32	9	7	0	0.55	No
4	388.41	72.45	3.33	9	7	0	0.55	No
5	332.35	85.22	2.62	7	6	2	0.55	No

Table 2. ADMET evaluation of five compounds (**1**-**5**) using pkCSM

Criteria	1	2	3	4	5
Caco-2 > 0.90 (log Papp in 10^{-6} cm/s)	1.068	1.054	1.088	1.088	1.062
Human Intestinal Absorption (+HIA > 30% and -HIA < 30%)	93.3	99.229	99.059	99.059	93.489
BBB permeability (+log BB > 0.30 and -log BB < -1.00)	-0.609	-0.092	-0.844	-0.844	-0.637
CYP2D6 substrate	No	No	No	No	No
CYP3A4 substrate	Yes	Yes	Yes	Yes	Yes
Total clearance (log mL/min/Kg)	0.09	-0.007	0.174	0.729	0.192
AMES toxicity	No	No	No	No	No
Hepatotoxicity	No	No	No	No	No
Skin Sensitization	No	No	No	No	No



A) RSMD: 0.887 Å B) RMSD: 1.652 Å C) RMSD: 0.883 Å

Figure 4. Validation of docking method with RMSD value ≤ 2 Å. A) Combrestatin A, B) Erlotinib, and C) AZD5438, with each redocked ligand (yellow color)

Table 3. Binding affinity and key interactions of **5** with three protein targets (tubulin, EGFR, and CDK2)

Protein	Binding Affinity (kcal/mol)			
	Combretastatin A4	Erlotinib	AZD5438	Compound 5
Tubulin (PDB ID: 5LYJ)	-7.1	-	-	-7.4
EGFR (PDB ID: 1M17)	-	-6.7	-	-7.3
CDK2 (PDB ID: 6GUE)	-	-	-9.2	-8.5

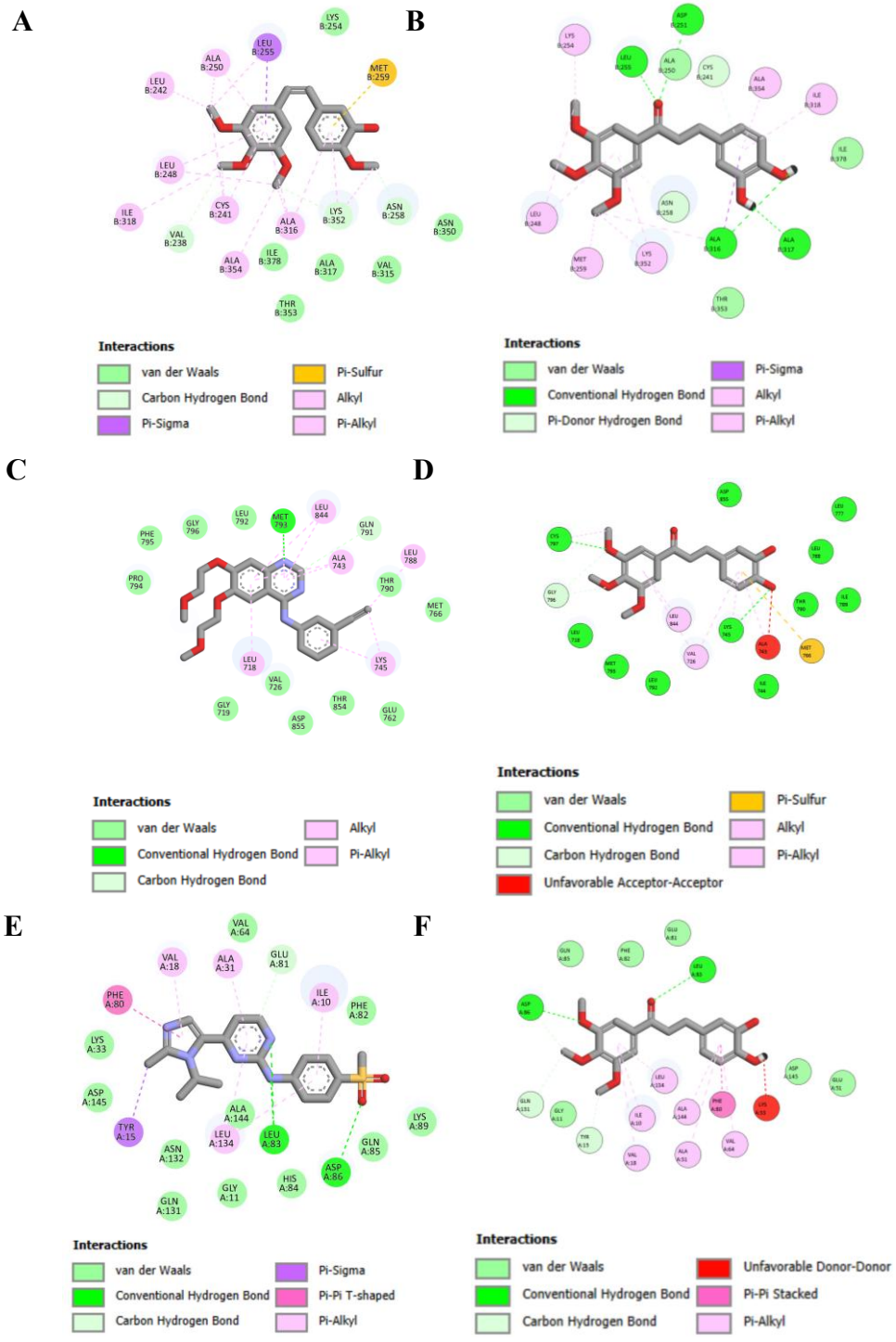
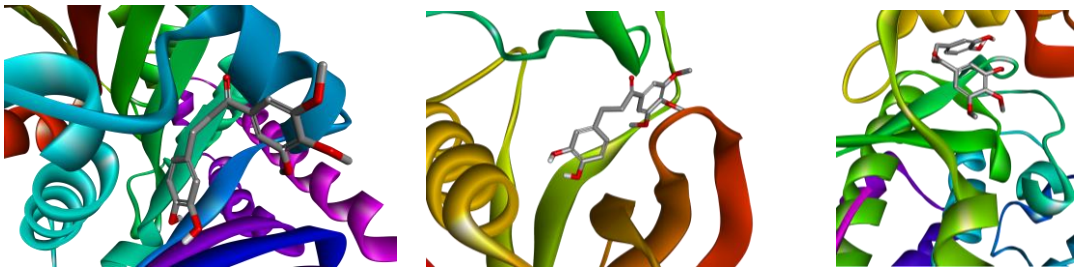


Figure 5. 2D interactions of ligand-protein complexes. A) Combretastatin A4-tubulin complex, B) compound **5**-tubulin complex, C) erlotinib-EGFR complex, D) compound **5**-EGFR complex, E) AZD5438-CDK2 complex, and F) compound **5**-CDK2 complex



A) Compound **5** with Tubulin B) Compound **5** with EGFR C) Compound **5** with CDK2

Figure 6. 3D structures of compound **5** with three protein targets (tubulin, EGFR, and CDK2)

To further our investigation, molecular docking was performed on compound **5** because it showed high potency against HCT-116 colon cancer cells without toxicity. This study aimed to predict the interaction between ligands and amino acid residues in the binding pocket by targeting overexpressed proteins in HCT-116 colon cancer cells. Various pharmacological strategies are used to manage colorectal cancer (CRC). One approach involves the administration of cytotoxic agents such as 5-fluorouracil, oxaliplatin, and irinotecan. An alternative strategy targets specific molecular pathways implicated in CRC. CRC predominantly affects epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF). Consequently, agents that inhibit these targets, such as cetuximab, bevacizumab, and ramucirumab, are commonly used in CRC therapy. Furthermore, overexpression of human cyclin-dependent kinase 2 (CDK2) has been observed in CRC patients, suggesting that the inhibition or downregulation of this kinase represents a viable therapeutic strategy [39]. Moreover, 3′,4′,5′-trimethoxychalcones inhibit tubulin by binding to colchicine-binding sites [40,41]. The docking method was validated by redocking each native ligand into the binding pocket of the protein with RMSD values ≤ 2 Å, as presented in **Figure 4**. Thus, compound **5** was docked into three protein targets, tubulin (PDB ID: 5LYJ), EGFR (PDB ID: 1M17), and CDK2 (PDB ID: 6GUE), to evaluate their potency and interactions using a molecular docking study.

Molecular docking results suggested that compound **5** exhibited a high binding affinity for CDK2 compared with tubulin and EGFR, with values of -8.5, -7.4, and -7.3 kcal/mol, respectively (**Table 3**). In addition, Beshr et al. reported that hybrids of chalcones possessing 8-hydroxyquinoline as dual inhibitors targeting tubulin and EGFR [42]. As shown in **Figure 5** (2D structure) and **Figure 6** (3D structure), compound 5 exhibited hydrogen bonding and hydrophobic interactions with three protein targets (tubulin, EGFR, and CDK2). Compound 5 showed four hydrogen bonding interactions with Asp251, Leu255, Ala316, and Ala317, including hydrophobic interactions in the binding pocket of tubulin, whereas combretastatin A4 displayed no hydrogen bonding interactions (**Figure 5A** and **5B**). Moreover, compound 5 formed two H-bonds with Cys797 and Lys745, π-sulfur with Met766, and hydrophobic interactions, whereas erlotinib only formed one H-bond with Met793 and hydrophobic interactions in the binding site of EGFR (**Figure 5C** and **5D**). Furthermore, compound 5 exhibited two H-bonds with Asp86 and Leu83, as well as π-π interactions with Phe80, which had similar interactions with the native ligand (AZD5438) in the binding pocket of CDK2, as shown in **Figures 5E** and **5F**. Therefore, this finding indicated that compound 5 had a strong interaction with CDK2 due to interaction with crucial amino acid residues in the active site.

4. CONCLUSION

Five synthesized 3′,4′,5′-trimethoxychalcones (**1**-**5**) from our previous work were evaluated for their cytotoxicity against human colorectal cancer cell lines (HCT-116) using an MTT assay. Four compounds (**1**, **3**, **4**, and **5**) exhibited strong inhibitory activity against HCT-116 colon cancer cells, with IC_{50} < 10 µM. Five compounds (**1-5)** were potent as drug candidates with no Lipinski violations and good pharmacokinetic profiles using SwissADME and pkCSM web servers. Compound **5** was docked against three protein targets (tubulin, EGFR, and CDK2). The binding affinities of compound **5** were -7.4, -7.3, and -8.5 kcal/mol, respectively, with dominant H-bond interactions in the binding pocket of the protein. Thus, this finding suggests that compound **5** has potential for further studies, including its inhibition mechanism, effectiveness *in vitro,* and *in vivo*.

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