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Cannabinoids as Promising Inhibitors of HER2-Tyrosine Kinase: A Novel Strategy for Targeting HER2-Positive Ovarian Cancer

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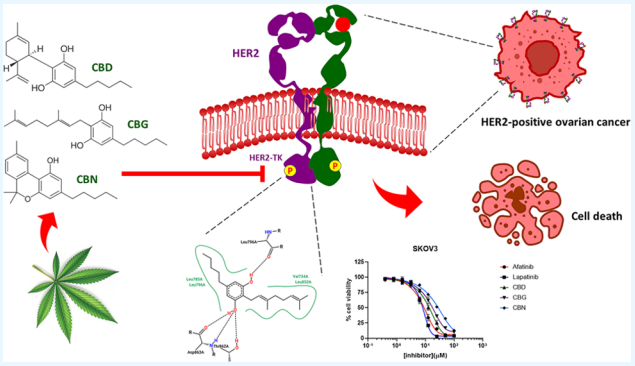
ABSTRACT: Human epidermal growth factor receptor 2 (HER2) is a transmembrane receptor within the ErbB family that plays a pivotal role in the progression of various aggressive cancers. HER2-positive tumors often develop resistance to standard therapies, necessitating the exploration of innovative treatment options. Cannabinoids, bioactive compounds from *Cannabis sativa* such as cannabidiol (CBD), cannabigerol (CBG), and cannabinol (CBN), have gained attention for their potential anticancer properties. This study evaluates the efficacy of CBD, CBG, and CBN in targeting HER2-positive ovarian cancer through kinase inhibition assays, surface plasmon resonance (SPR), molecular docking, and cell viability assessments. SPR analysis revealed that cannabinoids bind strongly to HER2-tyrosine kinase (HER2-TK), with CBD showing the highest affinity (K_D = 6.16 μM), significantly better than afatinib (K_D = 26.30 μM), and CBG demonstrating moderate affinity (K_D = 17.07 μM). In kinase inhibition assays, CBG was the most potent inhibitor (IC_{50} = 24.7 nM), followed by CBD (IC_{50} = 38 nM), suggesting their ability to disrupt HER2-mediated signaling pathways. Molecular docking studies highlighted critical interactions between cannabinoids and essential HER2 residues (Leu796, Thr862, Asp863). In cell viability assays, CBD and CBG effectively inhibited the growth of HER2-positive SKOV3 cells (IC_{50} = 13.8 μM and 16.6 μM, respectively), comparable to traditional tyrosine kinase inhibitors. These findings underscore the therapeutic potential of cannabinoids, particularly CBD and CBG, as alternative or adjunct therapies for HER2-positive cancers, with the promise of mitigating resistance and adverse effects associated with existing treatments.



1. INTRODUCTION

The quest for effective cancer therapies remains a major challenge, particularly in the case of human epidermal growth factor receptor 2 (HER2)-positive cancers, which encompass a substantial subset of aggressive breast and ovarian cancers. These cancers are characterized by the overexpression of the HER2, a transmembrane tyrosine kinase receptor that plays a critical role in cellular signaling pathways governing growth, differentiation, and survival.[1−3] HER2 overexpression occurs in approximately 20−30% of breast cancers and a significant subset of ovarian cancers, correlating with aggressive disease progression and poor prognosis.[4,5] Unlike other members of the EGFR family, HER2 is a ligand-independent receptor that frequently dimerizes with other receptors such as HER3, triggering downstream signaling pathways that contribute to oncogenesis, including the PI3K/Akt and MAPK/ERK pathways.[6−8] This receptor-driven signaling cascade fosters tumor proliferation and resistance to apoptosis, marking HER2-positive cancers as particularly challenging to treat.[9]

Current therapeutic strategies targeting HER2, such as monoclonal antibodies (*e.g.*, pertuzumab), antibody-drug conjugate (*e.g.*, trastuzumab emtansine (T-DM1)), and tyrosine kinase inhibitors (TKIs) (*e.g.*, lapatinib and afatinib), have provided significant clinical benefits. These agents disrupt HER2 signaling, thereby reducing tumor growth and improving patient survival.[10−12] However, despite initial successes, acquired resistance to these therapies is a recurring issue, often arising through mutations in HER2 or alternative activation of compensatory pathways that reestablish signaling in the cancer cells.[13−16] This resistance, coupled with potential side effects associated with traditional therapies, underscores an urgent need for novel agents with distinct mechanisms of

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action to target HER2-positive cancers effectively.[17] Emerging research suggests that cannabinoids, active compounds derived from *Cannabis sativa* L. (Cannabaceae), may represent promising alternatives or adjuncts to conventional HER2-targeted therapies. Phytocannabinoids such as CBD, CBG, and CBN are primarily known for their interactions with the endocannabinoid system but have been shown to influence various molecular pathways involved in cancer development.[18−20] These compounds modulate signaling pathways critical for cancer progression, including the PI3K/Akt and MAPK/ERK pathways, which are also implicated in HER2-driven signaling.[21,22] Interestingly, CBD has demonstrated cancer preventive activity in HER2-positive breast cancer models by inhibiting cell proliferation and inducing apoptosis, though the underlying mechanisms remain to be fully elucidated.[23,24] This mechanistic overlap between cannabinoid action and HER2 signaling highlights the potential of cannabinoids as alternative HER2 inhibitors, especially for cancers that have developed resistance to conventional TKIs.[25,26]

In this study, we investigate the binding kinetics and structural interactions of cannabinoids (CBD, CBG, and CBN) with HER2-TK using surface plasmon resonance (SPR) analysis. SPR is a powerful technique for real-time assessment of molecular interactions, allowing for precise quantification of binding affinities.[27,28] Our results reveal that CBD binds to HER2-TK with a dissociation constant (K_D) of 6.16 μM, significantly stronger than the clinically approved TKI afatinib (K_D = 26.30 μM), suggesting a high affinity of CBD for the HER2 receptor. Furthermore, CBG exhibits a potent inhibitory effect on HER2-TK, with an IC_{50} of 24.7 nM, which, while higher than lapatinib (IC_{50} = 5.0 nM), underscores the cannabinoid's potential as a selective inhibitor. These findings are particularly significant given the preferential inhibitory effects of both CBD and CBG on HER2-positive cancer cells (SKOV3) over HER2-negative cells (MCF-7), indicating a targeted action of cannabinoids on HER2-driven pathways.

Cannabinoid-mediated inhibition of HER2 presents a unique mechanism that could complement or enhance the efficacy of existing HER2-targeted therapies. Unlike traditional TKIs that directly target HER2-TK activity, cannabinoids offer broader biological effects, including the modulation of immune responses[29−31] and induction of autophagy,[32−34] which may help overcome resistance mechanisms in HER2-positive cancers. Additionally, cannabinoids are associated with a favorable safety profile, which could minimize adverse effects commonly observed with conventional cancer therapies, thereby improving the quality of life for patients.[35,36]

Our study reveals the significant potential of cannabinoids as HER2-TK inhibitors, highlighting their high binding affinity and selective inhibition of HER2-positive cancer cells. These findings suggest that cannabinoids may serve as valuable therapeutic agents for HER2-positive cancers, providing a novel approach for overcoming resistance to current HER2-targeted treatments. By advancing our understanding of cannabinoid interactions with HER2, this research opens up new avenues in oncology, supporting the integration of cannabinoid-based therapies in HER2-driven cancer management.

2. MATERIALS AND METHODS

2.1. Expression and Purification of the Recombinant HER2-TK. Procedures for preparing HER2-TK were followed in accordance with the guidelines provided in a previous study.[37] In brief, HER2-TK protein was expressed in *Escherichia coli* BL21(DE3) pLysS (Novagen, Germany) using a recombinant pCold I plasmid system (Addgene). The culture was grown in LB medium, induced with 0.5 mM IPTG at OD600−0.6, and incubated overnight at 16 °C for protein expression. Harvested cells were lysed, and the protein was initially purified using a DEAE-Sepharose ion-exchange column (Cytiva). The protein fraction was dialyzed against buffer (pH 7.4) and further refined using Resource Q column chromatography (Cytiva) on an FPLC system (GE ÄKTA FPLC), employing a linear gradient of elution buffer. Final polishing was achieved *via* size-exclusion chromatography with a Superdex 200 pg column (Sigma-Aldrich) in PBS (pH 7.4). The purified protein was characterized by SDS-PAGE to confirm its purity and molecular weight.

2.2. Compounds Preparation. Afatinib and lapatinib were purchased from LC laboratories (Woburn, MA). Pure isolated cannabinoids (CBD (purity ≥98%), CBG (purity ≥98%), and CBN (purity ≥97%)) were purchased from Mile High Laboratories (Broomfield, CO). Before being utilized, the compounds were kept at −80 °C as stock solutions in dimethyl sulfoxide (DMSO). In the biological assay, the stock solutions were diluted to the designated concentrations in the reaction buffer or culture medium, using a maximum DMSO concentration of 1% (v/v).

2.3. Surface Plasmon Resonance (SPR) Analysis. SPR is a powerful technique used to study biomolecular interactions in real-time. By measuring changes in the refractive index near a gold surface,[28] SPR can quantify binding kinetics and affinities between molecules.[38] The binding of cannabinoid compounds (CBD, CBG, CBN) and afatinib, a tyrosine kinase inhibitor, with HER2-TK were evaluated using an Open SPR 2-Channel Starter Pack R4.2 (Nicoya, Canada). The compounds were injected into the flow-channel at various concentrations (0−50 μM) and subsequently passed over the HER2-TK-immobilized Amine Sensor Chip (NECTEC, NSTDA, Thailand).[39] In brief, the EDC/NHS coupling HER2-TK (50 μg/mL) was immobilized on an Amine Sensor Chip at a rate of 20 μL/min in running buffer (PBS buffer (pH 7.4) with 5% DMSO) at a response unit (RU) of 1000−2000. To determine binding kinetics, a running buffer containing the compounds at various concentrations (0, 3.125, 6.25, 12.5, 25, and 50 μM) was loaded to the chip surface at a rate of 30 μL/min. The Trace Drawer 1.9.1 software (Ridgeview Instruments, Sweden) was used to analyze the sensorgrams. The binding was assessed through the association rate constant (k_a), dissociation rate constant (k_d), and equilibrium dissociation constant (K_D).

2.4. HER2-TK Inhibitory Activity Assay. The five pure compounds (afatinib, lapatinib, CBD, CBG, and CBN) were tested for their inhibitory activity against HER2-TK using the ADP-Glo Kinase Assay (Promega). The experimental process was performed according to the method described by the manufacturer. Lapatinib and afatinib (Woburn) were used as control kinase-inhibitor. The reaction mixture (25 μL) contained 5 μL of kinase buffer (20 mM Tris−HCl (pH 7.5), 20 mM MgCl2, 0.1 mg/mL BSA), 5 μL of 25 μM ATP, 5 μL of 12.5 μg/mL poly(Glu:Tyr) substrate, 5 μL of 1 ng/μL HER2-TK (diluted in kinase buffer), and 5 μL of various compounds concentrations. The reactions were carried out in a solid white, 384-well Greiner Bio-One Lumitrac plate, and incubated at room temperature for 1 h. The remaining ATP

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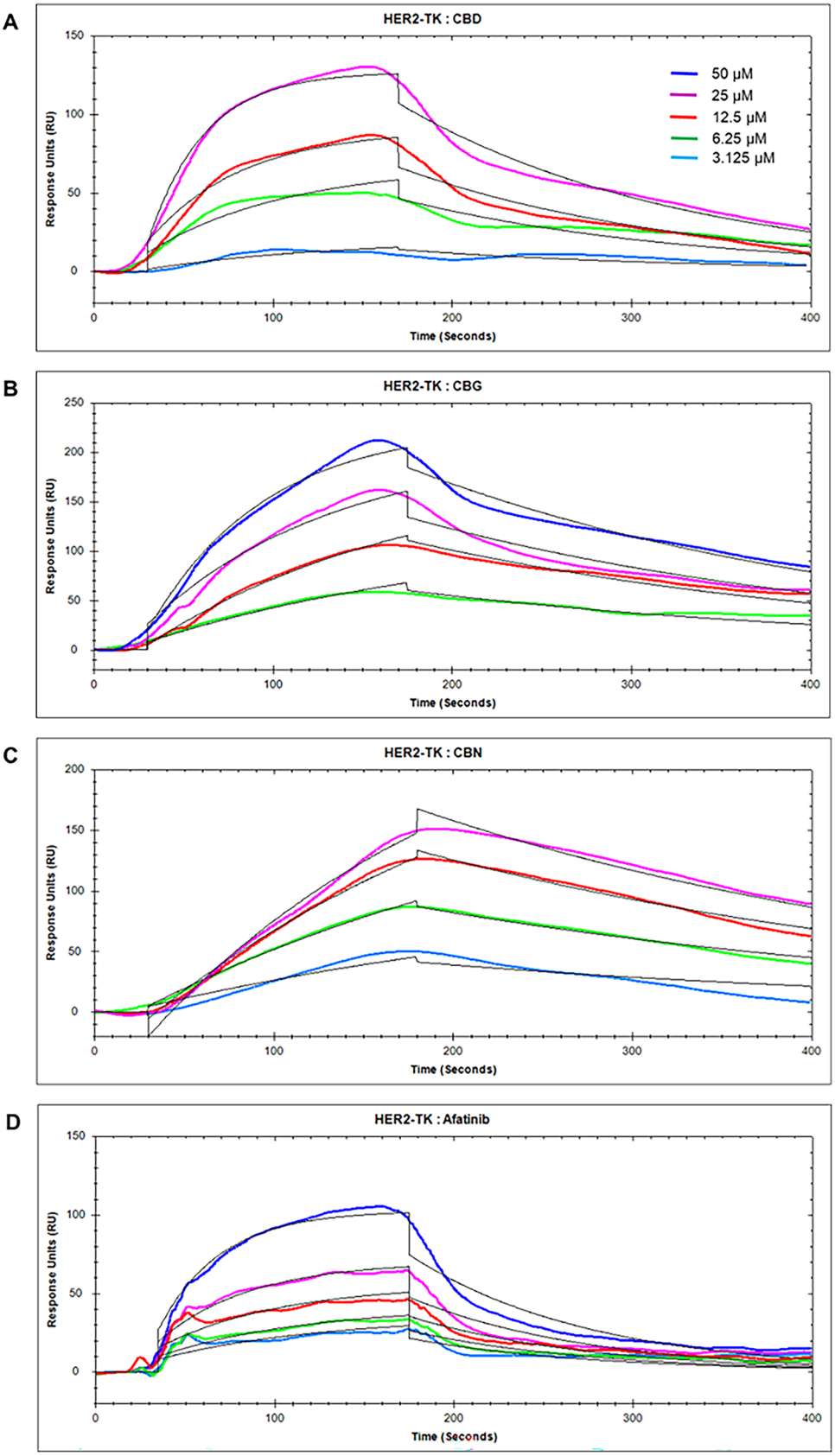

Figure 1. SPR analysis of binding between CBD (A), CBG (B), CBN (C), and afatinib (D) to HER2-TK. The 2-fold dilutions of compounds (at the indicated concentrations) were flown in running buffer at a rate of 30 μL/min. Sensorgrams were generated and analyzed using Trace Drawer 1.9.1 software (Ridgeview Instruments, Sweden). Colored lines represent the binding response signals at different concentrations of an analyte, and overlaid black lines represent fitted curves. The results, which are representative of three independent experiments, are expressed as mean ± SD (Table 1).

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was then depleted at room temperature for 40 min after the kinase reaction was terminated by the addition of 5 μL of the ADP-Glo reagent. Then, 10 μL of ADP-Glo detection reagent was added for 30 min to simultaneously convert ADP to ATP and to allow the newly synthesized ATP to be measured using a luciferase/luciferin reaction. Luminescence measurements were made using a microplate spectrophotometer (Synergy HTX Multi-Mode reader, BioTek). The IC_{50} values of inhibitors were determined using the nonlinear regression analysis of the log test compound concentration *versus* percentage control activity plots in GraphPad Prism (GraphPad Software Inc.). All assays were done in triplicate.

2.5. Molecular Docking. The three-dimensional (3D) crystal structure of human HER2 (erbb2) kinase domain (PDB: 3PP0) was retrieved from the PDB database and utilized as a reference geometry for molecular docking with its cocrystallized inhibitor (SYR127063).[40] The 3D structures of the ligands CBD, CBG, CBN, and the known FDA-approved drugs (lapatinib and afatinib) were downloaded from PubChem database.[41] These ligands were docked into the protein active site by using the Genetic Optimization for Ligand Docking (GOLD)[42] program based on the Kinase ChemScore (KCS) fitness function. The docking site was defined within 6 Å of the amino acids surrounding the reference inhibitor and run with a genetic algorithm 100 times without the early termination. The best docking poses were chosen based on the highest consensus scores from the gold scoring function and PLP. The protein−ligand interaction profiler (PLIP)[43] and BIOVIA Discovery Studio Visualizer V21.1.0.20299 were exploited to analyze the interaction of the best pose complexes. All protein structures and complexes were compared and graphically visualized using the PYMOL Molecular Graphics System, V2.5.2[44] and the PoseView of ProteinPlus server (https://proteins.plus).[45] Intermolecular interactions of the docked ligands on 3PP0-based HER2-TK model were observed and illustrated.[45]

2.6. Cell Culture and Cell Viability Assay. Cancer cells viability of HER2-positive cancer cells treated with cannabinoids was investigated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. SKOV3 (HER2-positive human ovarian cancer) and MCF-7 (HER2-negative cancer cells) were purchased from Biomedia (Thailand) Co., Ltd. SKOV3 was cultured as adherent monolayers in plastic tissue culture dishes in complete growth medium Dulbecco's Modified Eagle Medium (DMEM) (Gibco), while MCF-7 was cultured in Eagle's Minimum Essential Medium (EMEM) (Gibco) containing 10% fetal bovine serum (ATCC) and 100 U/mL antibiotics (Sigma-Aldrich). Before the experiment was set up, the cells were maintained at 37 °C in a humidified incubator with 5% CO_2 and were replenished every 3 days. Cells were seeded at a density of 1×10^4 cells/well in 96 well plates and incubated at 37 °C in 5% CO_2 for 16 h. The cells were then exposed to 100 μL of complete medium containing different concentrations of afatinib, lapatinib, CBD, CBG, and CBN (2-fold dilutions, starting at 100 μM) for 72 h. After incubation, the culture medium was replaced with 100 μL of MTT (ThermoFisher Scientific) solution and incubated at 37 °C for 3 h. The medium was then removed and 100 μL of DMSO was added to each well for solubilization of formazan. The absorbance of each well was measured using a spectrophotometer (BioTek Synergy HTX) with excitation and emission wavelengths of 550 and 590 nm, respectively. These experiments were done in triplicate. The IC_{50} values of inhibitors were calculated using the GraphPad Prism8 software (GraphPad Software Inc.) with a nonlinear regression analysis.

2.7. Statistical Analysis. The data were analyzed using GraphPad Prism8 software (GraphPad Software Inc.). Each experiment was conducted in triplicate, and the results are presented as the mean ± SD of three independent experiments.

3. RESULTS

3.1. Binding Affinity of Cannabinoids to HER2-TK. Surface plasmon resonance (SPR) analysis provided insights into the binding interactions between molecules, delivering valuable data on association and dissociation rates, as well as binding affinity.[38] In this context, we assess the binding of three cannabinoids (CBD, CBG, and CBN) and afatinib, a known TKI,[46] to HER2-TK. As shown in Figure 1, the parameters measured included the k_a, k_d, and K_D values, which were determined from the fits to at least three independents, normalized binding curves, calculated using the Trace Drawer 1.9.1 software (Ridgeview Instruments, Sweden). These measurements helped evaluate the effectiveness of these compounds as potential HER2-positive cancer therapies. Among the cannabinoids, CBD demonstrated the highest association rate constant (k_a) of 10.27×10^2 M^{-1} s^{-1} and the lowest K_D of 6.16 μM, suggesting the strongest and fastest binding to HER2-TK (Figure 1A and Table 1). Afatinib exhibited a k_a of 3.79×10^2 M^{-1} s^{-1} and a K_D of 26.30 μM, showing slower and less stable binding (Figure 1D and Table 1). CBG showed a moderate k_a of 2.21×10^2 M^{-1} s^{-1} and a K_D of 17.07 μM (Figure 1B and Table 1), while CBN had the lowest k_d of 3.14×10^{-3} s^{-1}, indicating the most stable binding but with a weaker K_D of 25.97 μM (Figure 1C and Table 1). The SPR results highlighted the significant potential of cannabinoids, especially CBD, as effective candidates for HER2-positive cancer therapy due to their high binding affinity and favorable kinetics. Its strong affinity to HER2-TK potentially bound to the ATP binding site and blocked its activity, leading to tumor growth suppression. CBG also showed a relatively good binding profile with a moderate k_a and k_d, and a better K_D compared to afatinib. This suggested that CBG could potentially be effective, though its affinity was lower than CBD (Table 1). CBG may have also served as a viable option but might have required higher concentrations or combination therapies to achieve comparable efficacy to CBD or current TKIs. CBN, while showing some potential due to its slower dissociation rate, may have been limited in its standalone effectiveness. Overall, these findings supported the potential of cannabinoids, particularly CBD, as HER2-targeted agents. However, further research was deemed essential to fully understand their therapeutic potential and to develop effective clinical applications.

Table 1. Compounds Binding Constants to HER2-TK Determined Using SPR Kinetic Analysis[a]

analytes	k_a (10^2/(M·s))	k_d (10^{-3}/s)	K_D (μM)
Afatinib	3.79 ± 0.10	9.95 ± 0.16	26.30 ± 0.26
CBD	10.27 ± 0.38	6.33 ± 0.06	6.16 ± 0.25
CBG	2.21 ± 0.11	3.77 ± 0.02	17.07 ± 0.80
CBN	1.22 ± 0.11	3.14 ± 0.09	25.97 ± 2.97

[a]Values are given as mean ± SD.

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3.2. HER2-TK Inhibition Potency of Cannabinoid Compounds. HER2-positive cancers were driven by the overexpression of the HER2 receptor, making them prime targets for TKIs such as afatinib and lapatinib.[47,48] Recently, cannabinoids like CBG, CBD, and CBN showed potential as anticancer agents.[49−52] This study compared the HER2-TK inhibition potency of these cannabinoids to that of afatinib and lapatinib, using IC_{50} values (the concentration required to inhibit 50% of the kinase activity) to assess their relative effectiveness (Figure 2). A lower IC_{50} value indicated a more

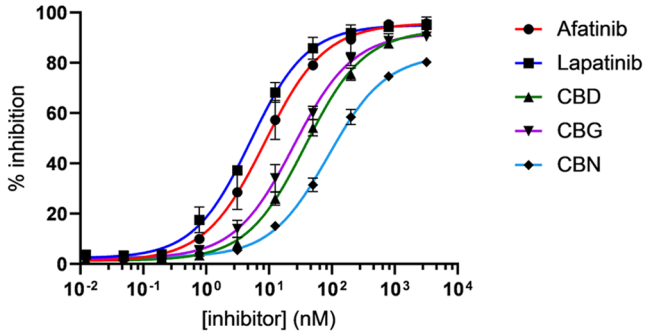

Figure 2. *In vitro* kinase inhibitory activity of cannabinoids (CBD, CBG, and CBN) and TKIs (lapatinib and afatinib) against HER2-TK. Graphs were plotted as percentage inhibition of kinase activity *versus* concentration of compounds, estimated using GraphPad Prism (GraphPad Software Inc.). IC_{50} values are given in Table 2, results are expressed as mean ± SD of triplicate experiments.

potent inhibitor. The study revealed that among the tested compounds, lapatinib and afatinib exhibited the highest potency, with IC_{50} values of 5.0 ± 2.6 and 8.6 ± 3.7 nM, respectively. Among the cannabinoids, CBG showed the most potent inhibition of HER2-TK with an IC_{50} of 24.7 ± 2.6 nM, followed by CBD at 38.0 ± 2.0 nM, and CBN at 87.4 ± 1.7 nM (Figure 2 and Table 2). This suggested that CBG might

Table 2. Summary of IC_{50} Values for *In Vitro* Kinase Inhibitory Activity against HER2-TK and Anticancer Activity against Cancer Cells of Cannabinoids[a]

		cell inhibition IC_{50} (μM)	
compounds	HER2-TK inhibition IC_{50} (nM)	MCF-7 (HER2-negative cell)	SKOV3 (HER2-positive cell)
Afatinib	8.6 ± 3.7	11.6 ± 0.6	8.4 ± 0.9
Lapatinib	5.0 ± 2.6	22.0 ± 0.5	8.0 ± 1.4
CBD	38.0 ± 2.0	24.1 ± 2.3	13.8 ± 1.7
CBG	24.7 ± 2.6	38.7 ± 1.4	16.6 ± 1.0
CBN	87.4 ± 1.7	44.9 ± 4.9	30.6 ± 2.8

[a]Values are given as mean ± SD.

have served as a promising lead compound for further optimization and development. Structural modifications or formulation strategies could have enhanced its potency, making it a viable candidate for targeted cancer therapies. Furthermore, these findings showed that although cannabinoids were not as potent as conventional TKIs, they still exhibited significant inhibitory activity against HER2-TK. Cannabinoids operated through different mechanisms of action and might have interacted with multiple signaling pathways,[53,54] potentially leading to synergistic effects when used in combination with traditional TKIs.[55,56] Moreover, cannabinoids were often associated with fewer side effects, which could have enhanced patient tolerance and compliance in long-term cancer therapy.[57,58]

3.3. Molecular Docking Reveals Molecular Interactions between HER2-TK and Cannabinoids. The binding affinities of the three cannabinoids were determined and compared with the known drugs using the GOLD docking program. The fitness score given by the consensus rescoring function showed that the best-docked pose of the reference ligand was 120.22 compared to lapatinib and afatinib, which scored 126.56 and 76.50, respectively (Table 3). With an

Table 3. Docking Scores of the Best-Docked Poses

ligand	GOLD score
SYR127063	120.22
lapatinib	126.56
afatinib	76.50
CBD	59.36
CBG	91.71
CBN	66.19

RMSD value of 2.7 Å, the docking pose orientation of the GOLD ligand was highly comparable to the native pose geometry of the crystallized ligand, differing mostly due to side chain flexibility (Figures 3A and 4A). The binding affinity of the best-docked pose of cannabinoids, as estimated by GOLD, showed that CBG had a higher docking score than CBD and CBN, which were 91.71, 59.36, and 66.19, respectively (Table 3 and Figure 3D−F). The two hydroxyl groups on the phenyl ring formed strong hydrogen bonds with Leu796, Thr862, and Asp863 in the range of 2.00−2.21 Å, which contributed significantly to CBG's binding to the HER2-TK active site (Figure 4B).

3.4. Cannabinoids Exhibit Inhibitory Effects on HER2-Positive Cancer Cells. HER2-positive cancer was a highly aggressive form of the disease characterized by the overexpression of HER2. This overexpression led to uncontrolled cell growth and proliferation.[59] Current treatments often involved targeted therapies like afatinib and lapatinib, which inhibited HER2 tyrosine kinase activity.[46,48] Although these TKIs demonstrated efficacy, their long-term use was often limited by the development of drug resistance and adverse effects,[16,60,61] necessitating the exploration of alternative therapeutic strategies. Cannabinoids, derived from the cannabis plant, showed promising anticancer effects in various studies.[18,19,21,49−52,57,58,62−64] However, their efficacy in comparison to established TKIs against HER2-positive cancers was still under investigation. This study aimed to evaluate the inhibitory effects of CBD, CBG, and CBN on HER2-positive ovarian cancer cell lines (SKOV3) compared to HER2-negative breast cancer cell lines (MCF-7) and benchmarked these effects against afatinib and lapatinib using IC_{50} values. The findings of this study indicated that cannabinoids, specifically CBD, CBG, and CBN, exhibited differential inhibitory effects on SKOV3 compared to MCF-7 (Figure 5A,B). Among the cannabinoids tested, CBD showed the most potent inhibitory effect on SKOV3 cells, with an IC_{50} value of 13.8 ± 1.7 μM, followed by CBG with an IC_{50} of 16.6 ± 1.0 μM. Both cannabinoids demonstrated greater efficacy in inhibiting SKOV3 cell proliferation compared to MCF-7 cells, where the IC_{50} values were 24.1 ± 2.3 μM for CBD and 38.7 ± 1.4 μM for CBG. CBN, although less effective than

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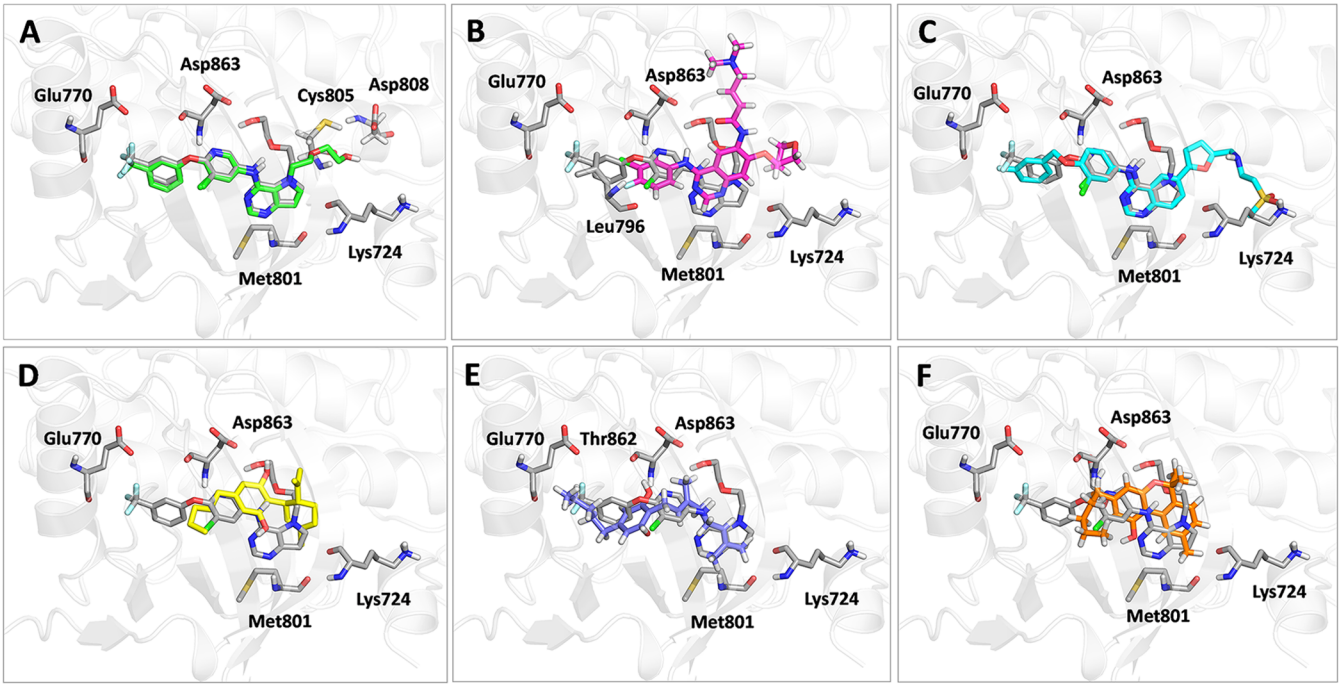

Figure 3. Molecular docking studies of HER2-TK (PDB: 3PP0) with reference and proposed inhibitors. The panels show the close view of SYR127063 (A), Lapatinib (B), Afatinib (C), CBD (D), CBG (E), and CBN (F) into active site of HER2-TK residues surrounded by hydrophobic contacts and hydrogen bonds. The gray lines represent the structures of interacting amino acids (with labels). The ligand structures are shown in stick models and represented by different carbon colors; the oxygen molecules are shown in red and the nitrogen molecules are shown in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

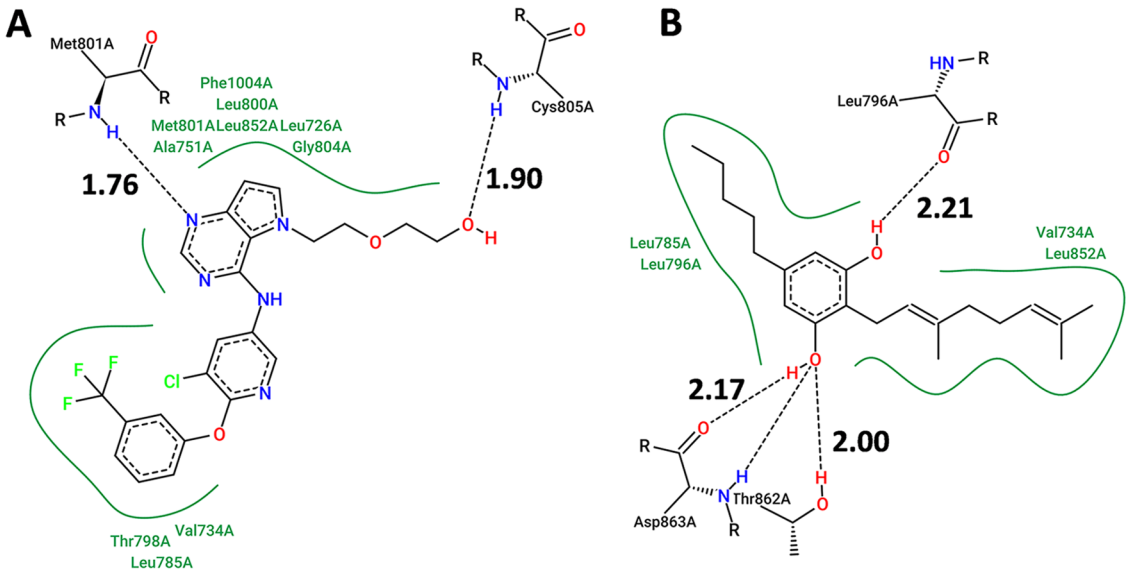

Figure 4. Orientation of SYR127063 (A) and CBG (B) in the active site of HER2-TK predicted by GOLD docking. The hydrogen bonds are shown in dotted lines, and distances (Å) between amino acids and corresponding ligand atoms are marked.

CBD and CBG, also exhibited a stronger inhibitory effect on SKOV3 cells (IC_{50} of 30.6 ± 2.8 μM) than on MCF-7 cells (IC_{50} of 44.9 ± 4.9 μM) (Table 2). These findings suggested that CBD and CBG showed moderate potential in inhibiting HER2-positive cancer cells, especially in SKOV3 ovarian cancer cells. Although they were less potent than HER2-TKIs (afatinib and lapatinib), they could have served as adjunctive agents in combination therapy to enhance overall treatment efficacy, potentially reducing the required dosage of conventional drugs and minimizing side effects. In addition, the IC_{50} of CBD for SKOV3 cells was closer to that of afatinib (8.4 ± 0.9 μM) and lapatinib (8.0 ± 1.4 μM), suggesting that CBD might have had potential as an alternative or complementary therapeutic agent in HER2-positive ovarian cancer treatment (Figure 5B and Table 2). Further studies were needed to elucidate the underlying molecular mechanisms and to assess the clinical viability of cannabinoids in cancer treatment strategies.

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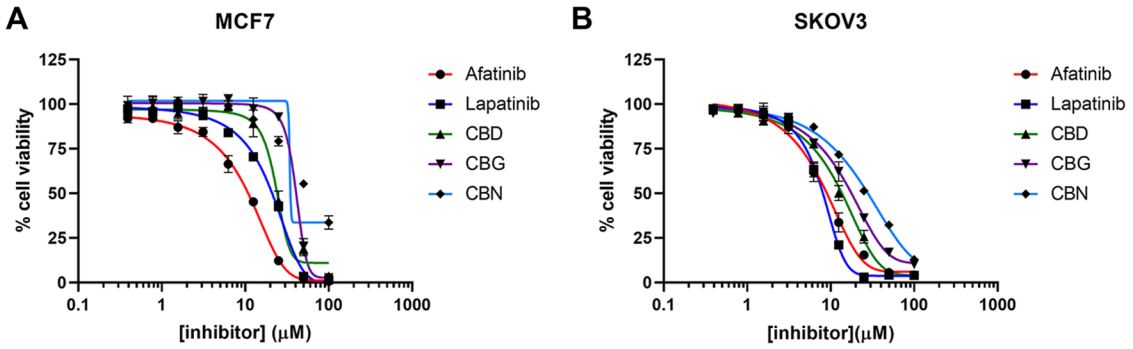

Figure 5. Growth inhibitory effects of afatinib and cannabinoids treatments in the MCF-7 (HER2-negative breast cancer) (A) and SKOV3 (HER2-positive human ovarian cancer) (B). The cells were treated with afatinib, lapatinib, CBD, CBG, and CBN for 3 days, and MTT reagent was added for the last 3 h. IC_{50} values are given in Table 2, results are expressed as mean ± SD of triplicate experiments.

4. DISCUSSION

This study presents novel findings on the application of cannabinoids as inhibitors of HER2-TK in treating HER2-positive ovarian cancer. Given the limitations and resistance issues associated with current HER2-targeted therapies, our results highlight exciting prospects for CBD, CBG, and CBN as alternative or adjunctive agents. By demonstrating strong binding affinities and kinase inhibition, particularly with CBD and CBG, this research underscores the potential for cannabinoids to serve as effective options for patients with HER2-positive ovarian cancers, potentially alleviating adverse effects commonly associated with traditional TKIs.

A significant finding in this study is the superior binding affinity of CBD to HER2-TK (K_D = 6.16 μM), which surpasses that of the TKI afatinib (K_D = 26.30 μM) (Figure 1 and Table 1). While afatinib and other TKIs like lapatinib are potent HER2 inhibitors, they are often limited by adverse effects, such as cardiotoxicity and interstitial lung disease.[61,65,66] By comparison, cannabinoids have shown lower systemic toxicities in previous studies, making them promising candidates for targeted therapies with reduced side effects.[67,68] This binding analysis study suggests that CBD and CBG could offer HER2-targeted efficacy, potentially comparable to or enhancing standard treatments, with an improved side effect profile.[69,70] Cannabinoids have previously demonstrated antiproliferative effects across several cancer cell types, often by modulating pathways such as PI3K/Akt and MAPK.[22,52,71] Molecular docking analysis revealed that CBG forms stable hydrogen bonds with key HER2-TK residues, such as Leu796, Thr862, and Asp863, contributing to its potent inhibitory capacity (Figures 3 and 4). These findings align with earlier studies showing the structural compatibility of cannabinoids with cancer-promoting pathways, highlighting cannabinoids' potential to disrupt HER2 activity and inhibit cancer cell growth selectively.[63,72,73]

Our results in cell viability assays further support cannabinoids as potential therapies for HER2-positive cancers. Both CBD and CBG inhibited the proliferation of HER2-positive SKOV3 ovarian cancer cells with IC_{50} values of 13.76 and 16.61 μM, respectively. These results are comparable to the IC_{50} values of conventional TKIs such as lapatinib (7.98 μM) and afatinib (8.37 μM), suggesting that cannabinoids might provide an alternative therapeutic route with fewer adverse effects[32,74] (Figure 5B and Table 2). In fact, our data support previous research that highlights CBD's role in sensitizing cancer cells to other treatments, potentially lowering the necessary doses of TKIs, thereby reducing their toxicity profiles.[62,75−77] Moreover, the potential for cannabinoids to address resistance mechanisms in HER2-positive cancers is particularly noteworthy. HER2-targeted therapies, such as trastuzumab, are highly effective initially but can eventually lead to drug resistance.[60,78,79] Cannabinoids, which exhibit multipathway interactions and low toxicity, may serve as adjunct therapies to help overcome resistance by modulating pathways critical to cancer cell survival.[71] For example, CBG's strong inhibitory effects on HER2-TK (IC_{50} = 24.7 nM) underscore its potential as a standalone or combination therapy (Figure 2 and Table 2), opening avenues for further exploration of cannabinoid efficacy when combined with conventional TKIs.[69,70]

This study not only aligns with but also expands on the current understanding of cannabinoids in cancer therapy by confirming their potential as HER2-TK inhibitors. Prior studies have shown that cannabinoids interact with various receptors and can be selectively effective against cancer cells.[68,80] The binding and structural analyses in this research add a new level of detail, revealing cannabinoids' high affinity and inhibitory potential on HER2-TK, which could represent a major breakthrough for HER2-positive ovarian cancer treatment.[67] The findings here reinforce that cannabinoid, especially CBD and CBG, have promise as therapeutic agents, whether used alone or in synergy with existing HER2-targeted treatments. Future research should focus on elucidating the precise mechanisms by which cannabinoids exert their effects, optimizing their use in combination therapies, and conducting clinical trials to validate their efficacy and safety in cancer treatment.

5. CONCLUSIONS

This study provides compelling evidence for the potential of cannabinoids, particularly CBD and CBG, as effective inhibitors of HER2-TK in HER2-positive ovarian cancer. The significant binding affinities and potent inhibitory effects observed in kinase assays and cell viability experiments highlight cannabinoids as promising candidates for alternative or adjunctive therapies, especially in patients facing challenges with conventional treatments. The findings not only indicate that cannabinoids can disrupt HER2-mediated signaling pathways but also suggest that they may enhance the therapeutic efficacy of existing TKIs while potentially mitigating associated side effects. The molecular docking analysis reinforces the understanding of how cannabinoids interact with critical residues in HER2-TK, revealing a detailed

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mechanism of action that warrants further exploration. Given the rising concerns regarding resistance to standard HER2-targeted therapies and their adverse effects, the incorporation of cannabinoids into treatment regimens may offer a novel and beneficial strategy for managing HER2-positive ovarian cancer.

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Author Contributions

T.L. designed and performed *in vitro* experiments. N.J. performed *in silico* experiment. T.L. and N.J. analyzed the data and wrote the original draft. T.L.V. and M.H. designed and fabricated the SPR sensor chip. S.S. supervised the experiment. T.L. and K.C. acquired the funds, discussed the results and contributed to the final manuscript. K.C. designed the study plan and supervised the project. All authors have read and agreed to the published version of the manuscript.

Notes

The authors declare no competing financial interest.

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