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Novel Anti-proliferative Application of Telmisartan and Candesartan: Synergistic Repurposing Strategy Against Colorectal Cancer

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ABSTRACT

Background: Colorectal cancer (CRC) remains a significant global health burden with high mortality and rising resistance to chemotherapy. Angiotensin receptor blockers (ARBs), traditionally used to treat hypertension, have recently been shown to have anti-cancer effects by inhibiting the angiotensin II type 1 receptor (AT1).
Objectives: To assess the anti-proliferative effects of telmisartan and candesartan on CRC cells and their synergy with chemotherapy.
Materials and methods: This in vitro study used CRC cell lines to assess viability (MTT assay), growth (soft agar), migration (wound healing), cell cycle and apoptosis (flow cytometry), and gene expression of B-cell lymphoma 2 (BCL2), vascular endothelial growth factor (VEGF), and AT1 by quantitative polymerase chain reaction.
Results: Telmisartan and candesartan inhibited CRC cell growth in a time- and dose-dependent fashion, with IC_{50} values ranging from 63 to 274 μM. When combined with doxorubicin or 5-fluorouracil, they reduced chemotherapy IC_{50} values by up to 11.6-fold, indicating synergistic cytotoxicity (CI < 1). Both ARBs suppressed wound closure by more than 60%, decreased colony formation by over 50%, induced G0/G1 cell cycle arrest (42% to 66%), and markedly increased apoptosis (54.4% with candesartan *vs.* 4.6% in controls). Additionally, gene expression analysis revealed more than a twofold downregulation of BCL2, VEGF, and AT1.
Conclusion: Telmisartan and candesartan show anti-cancer effects in CRC and synergise with chemotherapy, supporting their potential for repurposing.
Keywords: Colorectal cancer; Angiotensin receptor blockers; Candesartan; Telmisartan; Anti-proliferative.

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INTRODUCTION

Colorectal cancer (CRC) continues to be one of the most common and dangerous cancers globally, accounting for around 10% of all cancer diagnoses and nearly 900,000 deaths each year [1]. A recent Iraqi study also indicates a rising incidence of CRC, underscoring its growing role as a regional public health concern [2]. Although advances in screening and treatment have improved outcomes, the prognosis for late-stage CRC remains poor due to chemoresistance and systemic toxicity associated with agents like 5-fluorouracil and doxorubicin [3, 4]. This has intensified the search for more effective and better-tolerated therapies, with drug repurposing emerging as a cost-efficient strategy to accelerate oncologic drug development by identifying new indications for existing agents. Among repurposed

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candidates, angiotensin receptor blockers (ARBs) are widely used antihypertensives and have demonstrated promising anticancer activity [5]. ARBs exert their effects primarily by antagonizing the angiotensin II type 1 receptor (AT1), a component of the renin-angiotensin system (RAS) that regulates not only blood pressure but also cell proliferation, inflammation, and angiogenesis [6]. RAS activation is implicated in tumour progression and resistance to apoptosis [7], while AT1 blockade has shown anti-proliferative, pro-apoptotic, and anti-angiogenic effects across various tumour types, including prostate, lung, pancreatic, endometrial, and breast cancers [8, 9]. However, their mechanistic role and therapeutic benefit in CRC, especially in combination with cytotoxic chemotherapy, remain underexplored [10].

Among ARBs, telmisartan functions as a partial agonist at peroxisome proliferator-activated receptor gamma (PPAR-γ), a nuclear receptor associated with anti-proliferative and pro-apoptotic signalling pathways. In contrast, candesartan is a potent and selective AT1 receptor antagonist with a well-established cardiovascular safety profile [6]. Both agents have demonstrated anti-tumour activity in various malignancies; however, direct comparative studies evaluating their cellular effects specifically in CRC remain limited [10, 11].

Given their distinct molecular profiles, exploring the distinct effects of telmisartan and candesartan may provide insights into their therapeutic potential and synergistic value when combined with standard chemotherapeutic agents. The purpose of this study was to evaluate and compare the anti-tumour effects of these two ARBs on CRC cell lines by assessing their impact on proliferation, migration, colony formation, apoptosis, and cell cycle progression, as well as their synergy with 5-fluorouracil and doxorubicin.

MATERIALS AND METHODS

Study design

This research was an in vitro study that was conducted to evaluate the anticancer potential of ARBs, including telmisartan and candesartan cilexetil, individually and in combination with chemotherapeutic agents, against human CRC cell lines. The experiments were conducted in the laboratory facilities of the University of Jordan from June 2022 to September 2023.

Multiple assays were conducted, including 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, colony formation assays, wound healing assays, flow cytometry to detect apoptosis, cell cycle analysis, and quantitative polymerase chain reaction (qPCR) for gene expression profiling. Drug synergy was calculated with the Chou–Talalay combination index method.

Materials

Human CRC cell lines (HCT116, SW620, SW480, HT-29, and Caco-2) were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin streptomycin at 37°C in 5% CO_2. Telmisartan and candesartan cilexetil (Sigma-Aldrich), 5-fluorouracil and doxorubicin (Cayman Chemical), and mitomycin C (Sigma-Aldrich) were dissolved in dimethyl sulfoxide (DMSO) (final concentration $\leq$ 0.1%). MTT (Sigma-Aldrich), noble agar (Difco), and apoptosis detection kits (PI, RNase A, Annexin V-FITC; BD Pharmingen) were used for viability, colony, and apoptosis assays. Ribonucleic acid (RNA) was extracted using Direct-zol^{TM} (Zymo Research) and quantified via NanoDrop^{TM} 2000. Complementary deoxyribonucleic acid (cDNA) synthesis and qPCR were performed using Applied Biosystems kits on a 7900HT system.

Primers for the B-cell lymphoma 2 (BCL2), vascular endothelial growth factor (VEGF), AT1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were synthesised by IDT (Singapore); their sequences are presented in Table 1. Flow cytometry was performed using a BD FACSCanto^{TM} II and analysed with FlowJo^{TM}. Imaging was performed using EVOS^{TM} XL (Thermo Fisher); wound inserts from Ibidi (Cat. No. 80209). Data analysis was quantification was performed using GraphPad Prism 9.0. A combination index was calculated using GraphPad Prism 9.0, and a combination index analysis was conducted via CompuSyn. Image quantification was performed using ImageJ v1.53e.

MTT Assay

The anti-proliferative effects of candesartan and telmisartan on CRC cell lines were evaluated using the MTT assay. Cells (7,000 cells/well) were seeded in 96-well plates and treated with 25–200 µM drugs in $\leq$ 0.1% DMSO. Untreated wells with 0.1% DMSO served as controls. At 24-, 48-, and 72-hours post-treatment, 10 µL of MTT (5 mg/mL) was added. After incubation, the medium was removed, and formazan was dissolved in 100 µL DMSO. Absorbance at 570/630 nm was measured. Cell viability (%) was calculated relative to untreated controls (Equation 1). Experiments were done in triplicate.

$$\text{Cell viability}(\%) = \left(\frac{\text{Optical density of treated cells}}{\text{Optical density of untreated cells}}\right) \times 100\% \quad (1)$$

Drug Combination Assay

To assess the effects of candesartan or telmisartan alone or with 5-fluorouracil or doxorubicin on CRC cell lines (HCT116, SW620, Caco2), cells were seeded (7,000–10,000/well) in 96-well plates. Fixed-dose ratios based on IC_{50} values ensured equipotent combinations, followed by 24-hour treatment. Cell viability was assessed via MTT assay. The combination index (CI) was calculated using CompuSyn software based on the Chou-Talalay method (Equation 2). The Chou-Talalay Combination Index Theorem (CI) is expressed as follows:

$$\text{CI} = \frac{D_1}{Dx_1} + \frac{D_2}{Dx_2} \quad (2)$$

Where (Dx_1) is the dose of medication one that, when taken alone, produces 50% cell kill, and (D_1) is the dose of drug one that, when combined with (D_2), produces 50% cell kill. Drug 2 dosage to achieve 50% cell death alone is (Dx_2), while drug two dosage to achieve 50% cell death in conjunction with (D_1) is (D_2). CI values were interpreted as follows: CI $<$ 1 indicates synergism, CI = 1 denotes an additive effect, and CI $>$ 1 suggests antagonism.

Wound Healing Assay

Cells were seeded in 24-well plates with (Ibidi, Germany; Cat. No. 80209) inserts (30,000 cells/side) and incubated for 24 hours. After confirming adherence, inserts were removed, and cells were treated with mitomycin C (1μg/mL) to inhibit proliferation. After phosphate-buffered saline (PBS) wash, cells were treated with telmisartan or candesartan at IC_{50}

and $0.5 \times IC_{50}$. Wound closure was imaged at 0 and 48 hours and analysed using ImageJ software. The wound area at both time points was measured to calculate the percentage of closure using the formula (Equation 3 = Percentage of Wound Closure) below.

$$\%\,\text{Wound Closure} = \left(\frac{A_0 - A_{48}}{A_0}\right) \times 100\% \qquad (3)$$

Where: A_{texto} = wound area at time zero (0 hour), A_{48} = wound area after 48 hours of treatment.

Colony Formation Assay

A soft agar assay was performed to evaluate anchorage-independent growth. Six-well plates were prepared with a base layer of 1% noble agar in 2× DMEM, overlaid with a top layer containing 1×10^4 HCT116 cells resuspended in 0.6% agar. Cells were pretreated with candesartan or telmisartan at IC_{50} and sub-IC_{50} ($0.5 \times IC_{50}$) concentrations. Colonies were allowed to grow for 12 days at 37°C and then imaged using the EVOS XL Core microscope. The number of colonies per well and their average size were quantified using ImageJ software (version 1.53e).

Real-Time PCR (qPCR)

Caco2, HCT116, HT-29, SW620, and SW480 cells were seeded overnight. HCT116 cells were treated for 24 hours with 0.1 and 0.25 IC_{50} of telmisartan and candesartan; others remained untreated. RNA was extracted using the Direct-zol kit and assessed via NanoDrop (260/280 = 1.8–2.0) and gel electrophoresis. cDNA was synthesized, and qPCR was performed using SYBR Green on a 7900 system. Gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH normalization. Primer details are presented in Table 1. Equation 4: ΔΔCt Method for Relative Quantification of Gene Expression

$$\Delta Ct = CT_{\text{target gene}} - CT_{\text{housekeeping gene}}$$

$$\Delta\Delta Ct = \Delta Ct_{\text{treated sample}} - \Delta Ct_{\text{control sample}}$$

$$\text{Fold change} = 2^{-\Delta\Delta Ct}$$

Where: Ct = Cycle threshold, $Ct_{\text{target gene}}$ = Ct value of the gene of interest, $Ct_{\text{housekeeping gene}}$ = Ct value of the reference gene (e.g., GAPDH), ΔCt = Normalized Ct value for each sample, $\Delta\Delta Ct$ = Difference in ΔCt between treated and control samples, Fold change = Relative expression level of the target gene in treated samples compared to control.

Flow Cytometry

HCT116 cells were grown to confluence, treated with IC_{50} doses of candesartan or telmisartan for 24 hours, fixed in 70% cold ethanol, and stored at -20°C overnight. After PBS washing, cells were stained with propidium iodide (PI) (50μg/mL) and RNase A (5μg/mL) for 30 min in the dark. cell cycle distribution was analyzed using a BD FACSCanto II flow cytometer. For apoptosis analysis, HCT116 cells (4×10^5/well) were treated with 2× IC_{50} concentrations of drugs for 24 hours. The collected cells were stained with Annexin V-fluorescein isothiocyanate (FITC)/PI) and analysed using the same flow cytometer and software.

Statistical analysis

Data were analysed using GraphPad Prism 9.0. Two-way ANOVA assessed treatment effects, with results shown as mean ± standard deviation (SD); a P-value < 0.05 was considered significant. IC_{50} values were calculated by non-linear regression. Unpaired t-tests compared candesartan and telmisartan. CompuSyn software calculated the combination index (CI). ImageJ 1.53e measured wound area, colony size, and colony count.

RESULTS

The MTT assay showed that telmisartan and candesartan reduced CRC cell viability (SW620, SW480, HCT116, HT-29, Caco2) in a time- and dose-dependent manner over 24–72 hours. As shown in Table 2, both drugs decreased cell survival, with IC_{50} values confirming significant anti-proliferative effects (P-value < 0.05). Notably, candesartan showed significantly lower IC_{50} than telmisartan in HCT116 and Caco2 cells at 72 hours (P-value < 0.05).

To determine the additive effects of telmisartan or candesartan in combination with chemotherapy agents, HCT116, SW620, and Caco2 cells were treated for 24 hours with either 5-fluorouracil or doxorubicin alone, with each drug. As shown in Table 3, combination index (CI) analysis revealed synergistic effects ($CI < 1$) in all cases, which indicated enhanced anti-proliferative activity. Table 3 also presents CI values, fold reduction, combination ratios, and IC_{50} values for all treatments.

The IC_{50} and $0.5 \times IC_{50}$ concentrations of telmisartan and candesartan were utilized to assess how their therapy affected the migration of HCT116 cells. Figure 1 shows that after 48 hours of treatment, the untreated control had 90% wound closure (partial closure), whereas cells treated with telmisartan and candesartan significantly reduced wound closure, with average closure rates of approximately 38% and 42%, respectively, compared to 90% in the untreated control group, as indicated by the P-value of ≤ 0.0001 for both drugs' IC_{50} and sub-IC_{50} (0.5 IC_{50}).

HCT116 cancer cells were treated with either IC_{50} and $0.5 \times IC_{50}$ concentrations of candesartan and telmisartan for 24 hours to investigate the impact of these medications on the capacity of CRC cells to form colonies. The results demonstrated that either telmisartan or candesartan therapy reduced the mean colony size and number of HCT116 cells in comparison to the untreated control. Furthermore, Figures 2 and 3 illustrate the impact of both treatments on colony size and number, including quantitative bar graph comparisons and statistical significance.

Flow cytometric analysis revealed that incubation of HCT116 cells with IC_{50} concentrations of telmisartan and candesartan for 24 hours resulted in G0/G1 phase arrest. Specifically, the percentage of cells in the G0/G1 increased from 47.2% in the control to 65.8% with candesartan and 59.3% by telmisartan, indicating a reduced progression into, or a reduction in, the S and G2/M phases.

Annexin V-FITC/PI double labeling was used to examine how telmisartan and candesartan affected the viability of CRC cells by promoting necrosis or apoptosis, as seen in Figure 4A. A substantial increase in apoptosis was observed in HCT116 cells treated with twice the IC_{50} of telmisartan and candesartan for 24 hours. Quantitative analysis of the flow cytometry data revealed showed a significant rise in apoptotic HCT116 cells following candesartan treatment (54.4%

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Table 1. The forward and reverse sequences of primers, along with their ideal annealing temperature*.

Gene Symbol	Forward Primer (5'→3')	Reverse Primer (5'→3')	Annealing Temp (°C)
AT1	TGTGGACTGAACCGACTTTCT	GAACTCTCACTCCTGTTGCT	58
BCL2	TTGTGGCCCTTCTTTGAGTTGC	GGGTGCGGTTCAGGTACTCAGTCA	59
VEGF	CTACCTCCACCATGCCAAGT	GCAGTAGCTGCGCTGATAGA	59
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC	58

* Ta: Annealing temperature; AT1: The gene for Angiotensin II type 1 receptor; BCL2: The gene for B-cell lymphoma 2; VEGF: The gene for vascular endothelial growth factor; GAPDH: The gene for glyceraldehyde 3-phosphate dehydrogenase.

Table 2. The IC_{50} values for telmisartan and candesartan in HCT116, HT-29, Caco2, SW620, and SW480 at 24, 48, and 72 hours. The data displayed is $IC_{50} \pm$ SD. Statistical comparisons between the two drugs at each time point and within each cell line were performed using GraphPad Prism 9. * P-value ≤ 0.05; ** P-value ≤ 0.01; *** P-value ≤ 0.001; **** P-value ≤ 0.0001; ns (not-significant P-value > 0.05). μM: micromolar.

Cell Line	Time in hours	Candesartan IC_{50} (μM)	Telmisartan IC_{50} (μM)	P-value	Significance
SW620	24	120.15	113.75	0.0050	**
SW480	24	62.58	78.90	0.0306	*
HCT116	24	82.32	115.60	0.0017	**
HT-29	24	167.95	271.00	0.000007	****
Caco2	24	73.74	273.50	0.000002	****
SW620	48	101.50	113.25	0.0131	*
SW480	48	59.64	103.50	0.000039	****
HCT116	48	81.83	104.00	0.000088	****
HT-29	48	123.35	178.25	0.000006	****
Caco2	48	67.41	264.65	0.000001	****
SW620	72	100.00	108.50	0.0300	*
SW480	72	81.41	119.50	0.000006	****
HCT116	72	74.60	71.30	0.6159	ns
HT-29	72	73.90	102.21	0.000077	****
Caco2	72	63.00	239.60	0.000001	****

total apoptosis, representing the combined Q2 [late] and Q4 [early] quadrants) compared to the untreated control. While that of telmisartan is 17.7% total apoptosis in HCT116 cells (sum of Q2 [late] and Q4 [early] apoptotic populations), as illustrated in Figure 4B&C.

qPCR analysis was used to identify AT1 gene expression in untreated CRC cell lines: SW620, SW480, Caco2, HCT116, and HT-29. The results indicated that the AT1 gene was extremely overexpressed in HCT116 cells compared to the other cell lines (Figure 5A). To further evaluate the molecular activity of ARBs, HCT116 cells were treated with 0.1 and 0.25 $\times$ IC_{50} concentrations of telmisartan or candesartan for 24 hours, and the expression levels of BCL2, VEGF, and AT1 were analyzed by qPCR, with GAPDH used as the internal reference gene for normalization. Furthermore, the expression levels of BCL2, VEGF, and AT1 genes were analyzed. As illustrated in Figure 5B, both ARB treatments significantly repressed the expression of BCL2, VEGF, and AT1 genes compared to untreated controls. Candesartan reduced BCL2, VEGF, and AT1 expression by approximately 65%, 58%, and 60%, respectively, whereas telmisartan produced reductions of around 40%, 35%, and 38%.

DISCUSSION

ARBs exhibit anticancer activity by blocking the RAS, as shown in breast, prostate, endometrial, and pancreatic cancers [6–12]. Emerging evidence also suggests their potential benefit in CRC, where AT1 inhibition can suppress tumor angiogenesis, reduce pro-survival signalling, and enhance the efficacy of standard chemotherapeutic regimens [13]. This study demonstrates that candesartan and telmisartan exert significant anti-tumor effects in multiple CRC cell lines by inhibiting proliferation and migration, reducing colony formation, inducing G0/G1 cell cycle arrest, and downregulating key oncogenic and angiogenic markers (BCL2, VEGF, and AT1). Notably, candesartan exhibited a stronger pro-apoptotic effect than telmisartan, while both agents showed synergistic cytotoxicity with 5-fluorouracil and doxorubicin, markedly reducing the required chemotherapy doses.

Consistent with previous findings, both agents had anti-proliferative effects in SW620, SW480, Caco2, HT-29, and HCT116 cells, as previously described in their application to breast and prostate cancer cell lines [7, 14]. Among the cell lines tested, HCT116 appeared more responsive to both ARBs than Caco2, especially at later time points. This was evident from lower IC_{50} values, aligning with qPCR data showing higher AT1 receptor expression in HCT116, which may enhance drug efficacy. These findings highlight the importance of cell-specific molecular signatures in determining ARB sensitivity.

5-Fluorouracil remains a CRC treatment standard, though resistance and toxicity limit its efficacy [15]. Co-administration with telmisartan or candesartan produced synergistic effects (CI < 1.0), especially in HCT116 cells (CI ≈ 0.6), reducing required drug concentrations. This reflects variability in ARB response among CRC subtypes. Doxoru-

Table 3. Combination ratio, fold reduction, combination index, and the IC50 of 5-fluorouracil and doxorubicin alone and in combination with telmisartan or candesartan. NA: Not applicable, µM: micromolar, Chemo: Chemotherapy, ARB: Angiotensin receptor blocker.

Drug Combination	Combination ratio (Chemo: ARB)	IC_{50} (μM)	Fold reduction	CI
Cell line: SW620	Time: 24 hours			
Doxorubicin alone	N/A	26.7	N/A	N/A
Doxorubicin and Candesartan	1:05	4.52	6.0	0.36
Doxorubicin and Telmisartan	1:04	12.51	2.13	0.87
5-Fluorouracil alone	N/A	44.4	N/A	N/A
5-Fluorouracil and Candesartan	1:03	15.02	2.95	0.78
5-Fluorouracil and Telmisartan	1:03	12.18	3.64	0.62
Cell line: HCT116	Time: 24 hours			
Doxorubicin alone	N/A	5.70	N/A	N/A
Doxorubicin and Candesartan	1:15	3.10	1.83	0.90
Doxorubicin and Telmisartan	1:21	1.16	4.91	0.41
5-Fluorouracil alone	N/A	98.59	N/A	N/A
5-Fluorouracil and Candesartan	1:01	42.2	2.33	0.69
5-Fluorouracil and Telmisartan	1:01	27.9	3.53	0.48
Cell line: Caco2	Time: 24 hours			
Doxorubicin alone	N/A	86.85	N/A	N/A
Doxorubicin and Candesartan	1:05	7.48	11.6	0.62
Doxorubicin and Telmisartan	1:03	31.1	2.79	0.68
5-Fluorouracil alone	N/A	394.0	N/A	N/A
5-Fluorouracil and Candesartan	1:05	5.8	67.9	0.43
5-Fluorouracil and Telmisartan	1:01	57.8	6.81	0.35

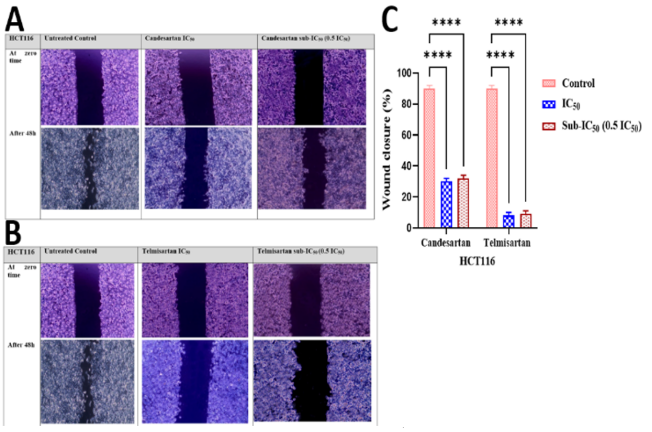

Figure 1. Cell migration in the wound healing assay. (A) Wound closure in HCT116 cells treated with IC_{50} and sub-IC_{50} concentrations of candesartan at 0 and 48 hours. (B) Wound closure in HCT116 cells treated with IC_{50} and sub-IC_{50} concentrations of telmisartan at 0 and 48 hours. (C) Quantitative analysis of wound closure percentage after 48 hours. Results are expressed as mean ± standard deviation (SD) from three independent experiments. Statistical significance levels are as follows: * P-value ≤ 0.05; ** P-value ≤ 0.01; *** P-value ≤ 0.001; **** P-value ≤ 0.0001.

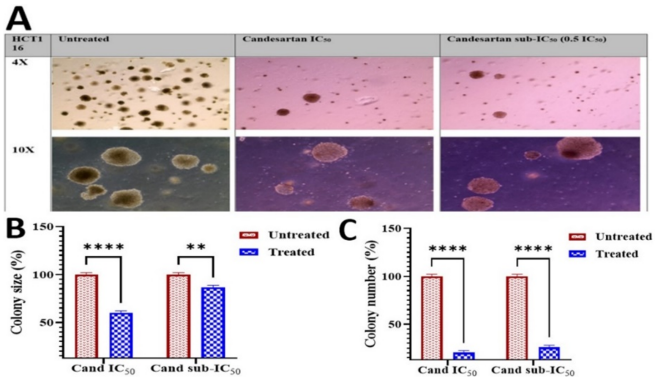

Figure 2. (A) Representative images of colony formation after 12-day treatment with candesartan (Cand) at sub-IC_{50} and IC_{50} concentrations, compared to untreated control. (B) Quantification of colony size in treated *vs.* untreated cells. (C) Quantification of colony number in treated *vs.* untreated cells. Statistical significance levels are as follows: * P-value ≤ 0.05; ** P-value ≤ 0.01; *** P-value ≤ 0.001; **** P-value ≤ 0.0001.

bicin, despite known toxicities, also showed enhanced cytotoxicity when combined with either ARB [16, 17]. From a clinical perspective, these findings suggest that repurposing ARBs as adjuvant agents could potentially allow for lower doses of chemotherapeutic drugs, thereby reducing treatment-related toxicity while maintaining or even enhancing anti-tumor efficacy. Such dose-sparing strategies are particularly relevant for patients with comorbid hypertension already receiving ARBs, offering a cost-effective and readily translatable approach pending confirmation in preclinical and clinical studies.

Regarding the wound healing assay, which is an early

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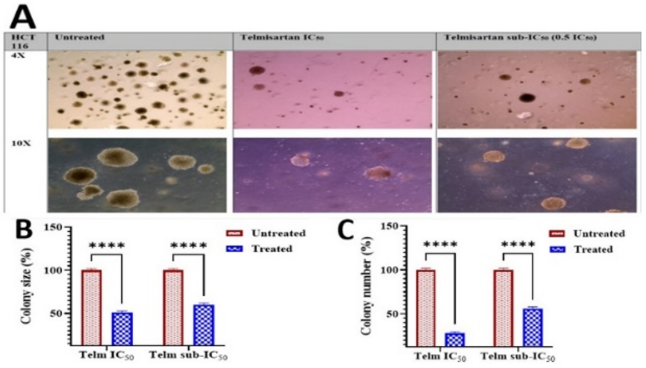

Figure 3. (A) Representative images of colony formation after 12-day treatment with telmisartan (Telm) at sub-IC_{50} and IC_{50} concentrations, compared to untreated control. (B) Quantification of colony size in treated *vs.* untreated cells. (C) Quantification of colony number in treated *vs.* untreated cells. Statistical significance levels are as follows: * P-value ≤ 0.05; ** P-value ≤ 0.01; *** P-value ≤ 0.001; **** P-value ≤ 0.0001.

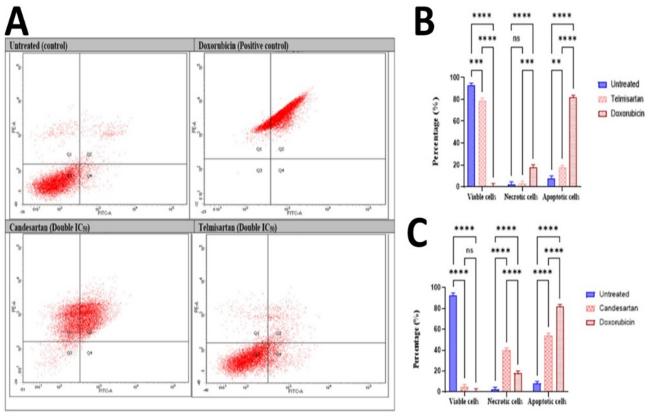

Figure 4. Apoptotic effects of ARBs on HCT116 cells as measured by Annexin V-FITC/PI staining. (A) Dot plot analysis showing distribution of viable (Q3), necrotic (Q1), late apoptotic (Q2), and early (apoptotic (Q4) HCT116 cells following 24-hour treatment with 2 × IC_{50} concentrations of candesartan and telmisartan. (B) Quantitative representation of the percentage of apoptotic cells (early + late apoptosis) induced by telmisartan compared to the untreated control. (C) Quantitative representation of the percentage of apoptotic cells (early + late apoptosis) induced by candesartan compared to the untreated control. Statistical significance levels are as follows: ns = not significant (P-value > 0.05); * P-value ≤ 0.05; ** P-value ≤ 0.01; *** P-value ≤ 0.001; **** P-value ≤ 0.0001.

method for studying 2-dimensional cell migration [18], it showed that telmisartan and candesartan significantly inhibited HCT116 cell migration at IC_{50} and sub-IC_{50} levels (P-value < 0.0001). In the colony formation assay, both drugs also markedly reduced colony size and number. This could be the first study reporting their impact on migration and colony formation in CRC cells.

Cancer-related genetic abnormalities disrupt cell cycle regulation, leading to uncontrolled growth. The cell cycle includes S (DNA replication), M (division), and gap phases G1 and G2 [19]. Telmisartan and candesartan (IC_{50}) induced G0/G1 arrest in HCT116 cells, indicating anti-tumor activity. This aligns with Oura et al., who reported G0/G1 arrest by telmisartan in hepatocellular carcinoma cells [19]. Clinically, induction of G0/G1 cell cycle arrest may translate into slowing tumor progression, increasing the window for therapeutic intervention, and potentially sensitizing cancer cells to other treatment modalities such as radiotherapy or deoxyribonucleic acid (DNA)-damaging agents. If validated in vivo, this mechanism could form part of a multi-targeted strategy to control tumor growth while minimizing systemic toxicity.

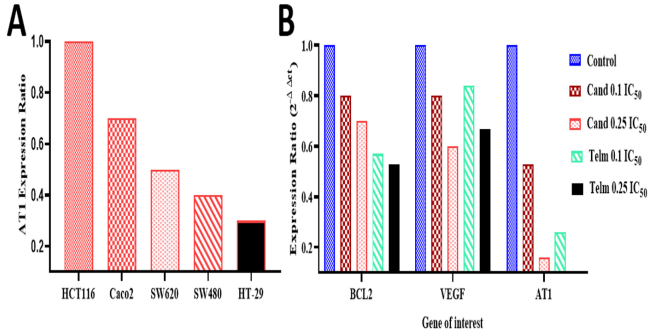

Figure 5. Effect of candesartan and telmisartan on gene expression in colorectal cancer (CRC) cells. (A) Relative AT1 gene expression across CRC cell lines (HCT116, SW620, SW480, Caco2, and HT-29), quantified by qPCR using the ΔΔCt method. (B) Gene expression changes of AT1, BCL2, and VEGF in HCT116 cells after 24-hour treatment with 0.1× and 0.25 × IC_{50} concentrations of candesartan (Cand) and telmisartan (Telm). Results are normalized to GAPDH and presented as fold change relative to untreated controls.

Apoptosis is a regulated process of programmed cell death essential for tissue homeostasis and cancer suppression [18]. In this study, treatment of HCT116 cells with twice the IC_{50} concentrations of candesartan and telmisartan for 24 hours induced significant apoptosis, reaching 54.4% and 17.7%, respectively, compared to 4.6% in untreated controls. These findings are consistent with previous studies showing telmisartan-induced apoptosis in other cancers, such as endometrial carcinoma [19]. From a clinical standpoint, the ability of ARBs to trigger substantial apoptosis in CRC cells suggests a potential role in eliminating resistant tumor cell populations, thereby reducing the likelihood of disease recurrence. This pro-apoptotic effect could complement existing chemotherapeutic regimens, particularly in patients with limited response to standard agents, and may be especially advantageous for those already receiving ARBs for cardiovascular indications.

The apoptotic response may result from downregulation of anti-apoptotic and pro-angiogenic genes. BCL2 and VEGF were both suppressed, suggesting involvement of mitochondrial apoptosis and reduced tumour angiogenesis. These changes enhance the anti-tumour effect of ARBs in CRC, potentially via AT1 and nuclear factor kappa B (NF-κB) modulation [20]. Further in vivo and pathway-specific studies are needed to confirm these findings.

Interestingly, the apoptotic response differed markedly between the two ARBs, with candesartan inducing a signifi-

cantly higher percentage of apoptosis (54.4%) compared to telmisartan (17.7%). This suggests that candesartan may exert a stronger pro-apoptotic influence in CRC cells, possibly due to more robust AT1 receptor antagonism. Conversely, telmisartan's partial PPAR-γ agonism may produce a more moderate apoptotic effect. This striking difference highlights the importance of considering drug-specific mechanisms in repurposing strategies for cancer treatment.

Exposure to 0.1 and 0.25 IC_{50} doses of candesartan and telmisartan downregulated BCL2 and AT1 gene expression in HCT116 cells, indicating pro-apoptotic activity via p53 modulation. Both ARBs also significantly reduced VEGF expression, a key angiogenesis regulator often upregulated in cancer [21], aligning with their observed anti-proliferative effects in CRC cells.

This study demonstrates that telmisartan and candesartan, two commonly prescribed ARBs, exert anti-tumor effects against CRC cell lines by suppressing proliferation and migration while promoting apoptosis. They also enhanced the cytotoxicity of standard chemotherapeutic agents, highlighting their potential as adjuvant therapies and candidates for drug repurposing in oncology. Nonetheless, the study has limitations. The findings are restricted to in vitro models, which do not fully replicate the tumor microenvironment or systemic drug responses. Furthermore, protein-level validation and detailed analysis of downstream signaling pathways were not conducted. Future research should include in vivo studies using animal models to evaluate efficacy and safety, as well as broader molecular profiling through proteomic and transcriptomic approaches. Exploring the effects of ARBs on immune regulation and tumor vasculature, alongside clinical studies in hypertensive CRC patients, may further clarify their therapeutic potential.

CONCLUSION

This study demonstrates that telmisartan and candesartan exert anti-tumor effects in CRC cells by reducing proliferation, migration, and colony formation, as well as by inducing G0/G1 cell cycle arrest. In combination with doxorubicin or 5-fluorouracil, they enhanced cytotoxicity and downregulated BCL2, VEGF, and AT1 expression. While these findings highlight their potential for drug repurposing in oncology, in vivo validation is required to assess efficacy, pharmacodynamics, and safety.

ETHICAL DECLARATIONS

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Ethics Approval and Consent to Participate

The study was approved by the Institutional Review Board of the University of Jordan (Approval Number 79/2022/3489; Dated 29 May 2022). Informed consent was not required for such kind of study.

Consent for Publication

Not applicable.

Availability of Data and Material

Data generated during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

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Authors' Contributions

R.S and T.M structured the concept; R.S and T.M. structured the methodology and chose the materials; A.F. and T.M conducted the data collection; R.S did the analysis and interpretation of data; R.M, T.M, A.F., and R.M. wrote the literature search; R.S, T.M, and R.M. conducted manuscript writing; R.S and R.M. conducted critical Reviews. All the authors read and approved the final version of the manuscript.

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