Journal of Dental Sciences 20 (2025) 1175–1195

Available online at www.sciencedirect.com

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journal homepage: www.e-jds.com

Original Article

Non-steroidal anti-inflammatory drugs (NSAIDs) regimens enhance synergistic selective anticancer efficacy of chemotherapeutic agents on cultured cells

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Received 15 January 2025; Final revision received 19 January 2025
Available online 18 February 2025

KEYWORDS
NSAIDs;
Novel regimens;
Synergism;
Anticancer;
Anti-efflux pump;
Antibacterial

Abstract *Background/purpose:* Cancer incidences are rising, presenting challenges due to severe side effects and resistance of chemotherapeutic agents. To address these issues, we propose a strategy involving pharmaceutical compositions of PTM (Phytopolyphenols, Targeting drugs, Metals) regimens. This study aimed to investigate NSAIDs (Non-steroidal anti-inflammatory drugs)-PTM regimens enhancing anticancer selectivity and efficacy of chemotherapeutic agents on cultured cancer cells.
Materials and methods: Effects of drugs on proliferation of cultured cancer cells and pathogens was assessed using MTT assay and optical density at 600 nm (OD600) respectively. Synergistic effects of drug combinations were determined using combination index and efficacy index. ATPase activity was assayed using a colorimetric method.
Results: NSAIDs-PTM regimens demonstrated selective and synergistic anticancer effects. They also enhanced anticancer selectivity and efficacy of Cisplatin, 5-Fluorouracil, and Methotrexate. The most effective NSAIDs-PTM regimens increased anticancer efficacy by 16, 4, and 23 fold against oral, lung, and colon cancer cell lines, respectively. Additionally, these NSAIDs-PTM regimens enhanced selective anticancer efficacy of Cisplatin, 5-Fluorouracil, and Methotrexate by 8–21 fold on the three cancer cells. Furthermore, all regimens exhibited synergistic anti-efflux pump ATPase activity and antibacterial effects against four cultured pathogens.
Conclusion: The findings indicate that NSAIDs-PTM regimens not only possess synergistic and selective anticancer and antibacterial properties but also enhance anticancer selectivity and efficacy of Cisplatin, 5-Fluorouracil, and Methotrexate. Notably, all regimens exhibited anti-efflux pump ATPase, which may help overcome multidrug resistance in cancer treatment. Given that all components of PTM regimens are clinically effective and safe, further clinical studies are warranted.

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https://doi.org/10.1016/j.jds.2025.01.019

Introduction

The global incidence of cancer continues to rise,[1] presenting significant challenges in treatment due to the serious side effects and the development of multi-drug resistance associated with various chemotherapeutic agents, such as cisplatin,[2–4] 5-fluorouracil,[5,6] and methotrexate.[7,8] Recent research has highlighted the potential of non-steroidal anti-inflammatory drugs (NSAIDs), including Celecoxib,[9,10] Diclofenac,[11,12] and Ibuprofen,[13,14] as effective anticancer agents. Beyond their role in inhibiting cyclooxygenase,[15,16] NSAIDs have been shown to engage additional mechanisms that contribute to their anticancer effects, involving pathways such as PPARγ, NFκB, and UPR.[17] However, the long-term use of NSAIDs is associated with a range of adverse effects, including cardiac dysfunction and hepatic and renal toxicities,[18–20] which necessitate urgent solutions.

Our proposed strategy to address these issues involves the development of pharmaceutical compositions that combine phytopolyphenols (P, such as curcumin and green tea polyphenols) with targeted drugs (T, including NSAIDs, cisplatin, 5-fluorouracil, and methotrexate) and metals (M, such as zinc sulfate), collectively referred to as PTM regimens. These PTM regimens exhibit pleiotropic and synergistic pharmacological effects, encompassing anti-inflammatory, antioxidant, anticancer, antibacterial, neuroprotective, and immunomodulatory properties.[21–26] Consequently, they have been recognized as innovative and effective approaches for the prevention and management of chronic diseases, including various cancers and infectious diseases,[27–29] as well as conditions such as dementia,[30] diabetes, and pain.[31]

In this study, we aimed to investigate the synergistic anticancer and antibacterial effects of NSAIDs-PTM regimens on cultured cancer cell lines (OECM-1, an oral squamous cell carcinoma; A549, a non-small cell lung cancer; and DLD-1, a colon cancer) and four bacterial pathogens (*Porphyromonas gingivalis*, *Streptococcus mutans* UA159, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). Additionally, we will explore how NSAIDs-PTM regimens can enhance the anticancer selectivity and efficacy of three chemotherapeutic agents (cisplatin, 5-fluorouracil, and methotrexate). The findings from this research suggest that NSAIDs-PTM regimens may represent promising therapeutic strategies for the prevention and management of cancers and infectious diseases, warranting further preclinical and clinical investigations.

Materials and methods

Drugs studied

Curcumin (C) was purchased from Merck Co. (Darmstadt, Germany). Green tea polyphenols(G), purified from Camellia sinensis and containing 98 % polyphenols (75 % catechins by HPLC and 50 % EGCG by HPLC), similar to polyphenol E,[38] were obtained from Hunan Huacheng Biotech, Inc. (China). Cisplatin(Cis) and thioridazine(TRZ) were sourced from Sigma Chemical Company (St. Louis, MO, USA). NSAIDs, 5-Fluorouracil(5-FU) and Methotrexate were acquired from the Department of Pharmacy at Chung-Shan Medical University Hospital (Taichung, Taiwan).

Cell culture

Cell culture medium (RPMI) and supplements were purchased from Sigma (St. Louis, MO, USA). All culture media were supplemented with heat-inactivated fetal bovine serum (FBS) from either JRH Biosciences or Hyclone, Thermo Scientific. SG (gingival epithelial cells), OECM-1 (oral squamous cell carcinoma cells), A549 (non-small cell lung cancer cells), and DLD-1 (colon cancer cells) were cultured in a CO_2 incubator at 5 % concentration and a temperature of 37 ± 0.5 °C.

The culture of pathogens

The anaerobic pathogen *Porphyromonas gingivalis* (P.g., ATCC 33277) was cultured in Luria–Bertani (LB) broth (Becton Dickinson, Sparks, MD) supplemented with hemin (5 μg/ml) and menadione (1 μg/ml).[3] *Streptococcus mutans* (S.m., UA159, ATCC 700610) was cultured in Brain Heart Infusion (BHI) broth (Becton Dickinson, Sparks, MD) at 37 ± 0.5 °C in an anaerobic chamber containing 10 % H_2, 5 % CO_2, and 85 % N_2 (Forma Scientific Inc., Marietta, OH, USA). *Pseudomonas aeruginosa* (P.a., ATCC 39327) was cultured in LB, and *Staphylococcus aureus* (S.a., ATCC 33591) was cultured in BHI at 37 ± 0.5 °C in aerobic conditions.

Anticancer and antibacterial potencies (IC50)

Inhibition of cultured cell growth and biofilm formation of pathogens were assessed using the MTT assay and optical density (OD600), respectively.[32]

The effects of the drugs on cells were evaluated using the MTT assay after the addition of various concentrations of drugs (10 μL/well) to 100 μL/well of cultured cells in a 96-well microplate for 72 h. The cells were then treated with MTT, and the absorbance was measured using a microplate reader at OD550.

The effects of the drugs on pathogens were evaluated by measuring the changes in optical density at 600 nm (OD600) after the addition of various drug concentrations (10 μL/well) to 100 μL/well of cultured bacteria. The bacteria were diluted with culture broth: *P. gingivalis* (P.g.) in LB supplemented with hemin and menadione, *Streptococcus mutans* (UA159) in brain heart infusion (BHI) supplemented with 0.2 % sucrose, *Pseudomonas aeruginosa* (P.a.) in LB, and

Staphylococcus aureus (S.a.) in BHI supplemented with 10 % human plasma, all in a 96-well microplate. The drug effects were assessed after 48 h of incubation with the bacteria by measuring the changes in OD600, which were then calculated as a percentage of the control treated with vehicle saline. The IC50 (the concentration required for 50 % inhibition) was determined from the concentration—inhibition curve.

Assay of ATPase activity

ATPase activities were assessed using a colorimetric method as previously described.[33] Briefly, after treatment with drugs for 72 h, the cells were incubated with the substrate ATP for 15 min at 37 °C ± 0.5°. The released inorganic phosphate (Pi) was quantified by measuring the optical density at 630 nm (OD630) after a 10-min reaction with malachite green-

Table 1 Anticancer (A), efflux pump ATPase inhibition (B) and antibacterial (C) effects of drug alone on three cultured cancer cells and four pathogens.

A. Anticancer effect				
Drugs	IC50 (μg/ml; Zn^{2+},μM)			
	SG	OECM-1	A549	DLD-1
C,Curcumin	215.9 ± 28.1	195.2 ± 30.3▲	137.9 ± 24.9▲	82.3 ± 10.9▲
G, Green tea polyphenols	176.4 ± 28.1	218.4 ± 14.6	182.6 ± 20.9	178.0 ± 19.9▲
G·C	98.1·98.1	49.5·49.5▲	58.3·58.3▲	48.8·48.8▲
ZnSO4	267.8 ± 7.9	405.1 ± 15.9	561.80 ± 12.8	295.0 ± 19.8
Cl,Celecoxib	58.2 ± 3.4	84.5 ± 9.0	32.2 ± 1.4▲	129.8 ± 3.1
Dn,Diclophenac	40.5 ± 3.1	44.5 ± 0.8	53.9 ± 1.8	52.7 ± 6.7
Ibu,Ibuprofen	>150	>150	>150	>150
Cis,Cisplatin	6.5 ± 0.4	5.8 ± 0.5▲	10.1 ± 0.8	8.39 ± 1.0
5FU,5-Fluorouracil	3.1 ± 0.3	4.3 ± 0.3	3.5 ± 0.3	3.64 ± 0.3
MTX,Methotrexate	0.018 ± 0.009	0.023 ± 0.009	0.061 ± 0.008	0.044 ± 0.011
B. Efflux pump ATPase inhibition				
Drugs	IC50 (μg/ml; Zn^{2+},μM)			
	SG	OECM-1	A549	DLD-1
C,Curcumin	33.8 ± 2.3	58.7 ± 7.4	323.6 ± 15.6	200.6 ± 23.5
G, Green tea polyphenols	80.8 ± 2.4	50.8 ± 2.5▲	66.1 ± 4.5	58.5 ± 3.0▲
G·C	62.2·62.2	53.3·53.3▲	75.0·75.0	69.2·69.2
ZnSO4	321.5 ± 36.5	396.9 ± 30.2	426.1 ± 43.0	299.1 ± 36.6▲
Cl,Celecoxib	194.6 ± 9.5	498.2 ± 38.4	434.7 ± 39.1	185.6 ± 8.6▲
Dn,Diclophenac	51.7 ± 2.7	99.9 ± 8.5	108.2 ± 4.9	90.1 ± 4.1
Ibu,Ibuprofen	>150	>150	>150	>150
Cis,Cisplatin	2.6 ± 0.9	3.3 ± 0.6	4.6 ± 1.3	3.9 ± 0.5
5FU,5-Fluorouracil	5.2 ± 0.3	10.8 ± 1.1	4.0 ± 0.2▲	4.4 ± 0.2▲
MTX,Methotrexate	0.15 ± 0.005	0.59 ± 0.022	0.13 ± 0.004▲	0.17 ± 0.005
C. Anti-biofilm formation				
Drugs	IC50 (μg/ml; Zn^{2+},μM)			
	P.g.	UA159	P.a.	S.a.
C,Curcumin	122.9 ± 7.9★	251 ± 9.5	602.3 ± 11.3	238.3 ± 11.3
G, Green tea polyphenols	125 ± 12.7★	446.5 ± 18.5	183.9 ± 27.8	>1000
G·C	92.7 ± 7.0·92.7 ± 7.0★	95.5 ± 4.5·95.5 ± 4.5	149 ± 9·149 ± 9	132.5 ± 3.5·132.5 ± 3.5
$ZnSO_4$	501.0 ± 41.6★	1858.3 ± 72.5	>3000	2035.4 ± 229
Cl,Celecoxib	589.3 ± 18.7	17 ± 1.1★	>1000	673.5 ± 3.7
Dn,Diclophenac	159.8 ± 3.2	39 ± 1.3★	>1000	35.7 ± 1.5★
Ibu,Ibuprofen	>100	>100	>100	>100
Cis,Cisplatin	44.9 ± 3.4	>100	17.4 ± 2.3★	>100
5FU,5-Fluorouracil	2840.8	9.7 ± 0.14★	49 ± 2	223.0 ± 20.8★
MTX,Methotrexate	30.7 ± 1.7	5.6 ± 1.3★	>100	>100

SG, normal gingival epithelium cell; OECM-1, oral squamous cell carcinoma; A549, lung cancer cell; DLD-1, colon cancer cell. P.g.,*Porphyromonas gingivalis*; UA159, *Streptococcus mutans*; P.a., *Pseudomonas aeruginosa*; S.a., *Staphylococcus aureus*.
IC50, half maximal inhibitory concentration.
▲Selective inhibitors with IC50 less than those of normal gingival epithelial cells SG.
★The most sensitive pathogen to the drug alone.

Table 2 Phytopolyphenols and $ZnSO_4$enhanced synergistic and selective anticancer (A), anti-efflux pump ATPase (B) and antibacterial (C) effects of NSAIDs on cultured cells and pathogens.

A. Anticancer effects								
Regimens	SG				OECM-1			
	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
CCl	9.1 ± 0.7·9.1 ± 0.7	0.2	23.7	6.4	9.8 ± 2.1·9.8 ± 2.1	0.2	19.9	8.6★
GCl	20.7 ± 2.6·20.7 ± 2.6	0.5	8.5	2.8	42.5 ± 12.6·42.5 ± 12.6	0.7	5.1	2.0
GCCl	14.3 ± 1.9·14.3 ± 1.9·14.3 ± 1.9	0.4	6.9	4.1	21.1 ± 8.4·21.1 ± 8.4·21.1 ± 8.4	0.7	2.3	4.0
ClZn	32.8 ± 7.3·10.9 ± 2.5	0.6		1.8	>150·50	>1.9		<0.6
CClZn	18.3 ± 1.2·18.3 ± 1.2·6.1 ± 0.4	0.4	11.8	3.2	21.1 ± 3.1·21.1 ± 3.1·7.0 ± 1.0	0.4	9.3	4.0
GClZn	18.8 ± 2.0·18.8 ± 2.0·6.3 ± 0.7	0.5	9.4	3.1	36.6 ± 6.6·36.6 ± 6.6·12.2 ± 2.2	0.6	6.0	2.3
GCClZn	12.9 ± 0.8·12.9 ± 0.8·12.9 ± 0.8·4.3 ± 0.3	0.4	7.6	4.5	21.0 ± 2.4·21.0 ± 2.4·21.0 ± 2.4·7.0 ± 0.8	0.7	2.4	4.0
CDn	29.8 ± 1.1·29.8 ± 1.1	0.9	7.2	1.4	29.6 ± 1.2·29.6 ± 1.2	0.8	6.6	1.5
GDn	24.3 ± 0.7·24.3 ± 0.7	0.7	7.3	1.7	43.0 ± 6.1·43.0 ± 6.1	1.2	5.1	1.0
GCDn	21.5 ± 0.7·21.5 ± 0.7·21.5 ± 0.7	0.8	4.6	1.9	25.1 ± 3.2·25.1 ± 3.2·25.1 ± 3.2	1.1	2.0	1.8
DnZn	54.6 ± 4.6·18.2 ± 1.5	1.4		0.7	53.9 ± 3.5·18.0 ± 1.2	1.3		0.8
CDnZn	22.5 ± 1.1·22.5 ± 1.1·7.5 ± 0.4	0.7	9.6	1.8	21.0 ± 0.4·21.0 ± 0.4·7.0 ± 0.1	0.6	9.3	2.1
GDnZn	31.2 ± 1.6·31.2 ± 1.6·10.4 ± 0.5	1.0	5.7	1.3	37.1 ± 1.3·37.1 ± 1.3·12.4 ± 0.4	1.0	5.9	1.2
GCDnZn	26.3 ± 1.1·26.3 ± 1.1·26.3 ± 1.1·8.8 ± 0.4	1.0	3.7	1.5	20.8 ± 1.2·20.8 ± 1.2·20.8 ± 1.2·6.9 ± 0.4	0.9	2.4	2.1★
CIbu	19.4 ± 2.3·9.7 ± 1.2	0.2	11.1	15.5	18.7 ± 2.7·9.4 ± 1.4	0.2	10.4	16.0★
GIbu	42.4 ± 3.2·21.2 ± 1.6	0.4	4.2	7.1	29.0 ± 2.·14.5 ± 1.0	0.2	7.5	10.3★
GCIbu	29.5 ± 0.8·29.5 ± 0.8·14.7 ± 0.4	0.4	3.3	10.2	25.9 ± 2.3·25.9 ± 2.3·13.0 ± 1.2	0.6	1.9	11.5★
IbuZn	>75·50	>0.7		<2.0	>75·50	>0.6		<2.0
CIbuZn	25.7 ± 3.1·12.9 ± 1.5·8.6 ± 1.0	0.2	8.4	11.6	26.2 ± 2.8·13.1 ± 1.4·8.7 ± 0.9	0.2	7.5	11.5★
GIbuZn	47.8 ± 4.6·23.9 ± 2.3·15.9 ± 1.5	0.5	3.7	6.3	54.7 ± 3.6·27.4 ± 1.8·18.2 ± 1.2	0.5	4.0	5.5
GCIbuZn	35.2 ± 3.1·35.2 ± 3.1·17.6 ± 1.5·11.7 ± 1.0	0.5	2.8	8.5	31.3 ± 1.5·31.3 ± 1.5·15.7 ± 0.8·10.4 ± 0.5	0.8	1.6	9.6★
Regimens	A549				DLD-1			
	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
CCl	11.7 ± 1.1·11.7 ± 1.1	0.4	11.8	2.8	6.0 ± 0.6·6.0 ± 0.6	0.1	13.7	21.6★
GCl	8.0 ± 0.3·8.0 ± 0.3	0.3	22.8	4.0★	22.3 ± 4.4·22.3 ± 4.4	0.3	8.0	5.8
GCCl	14.4 ± 4.8·14.4 ± 4.8·14.4 ± 4.8	0.7	4.0	2.2	17.9 ± 5.0·17.9 ± 5.0·17.9 ± 5.0	0.5	2.7	7.3
ClZn	27.0 ± 13.2·9.0 ± 4.4	0.9		1.2	120.9 ± 22.8·40.3 ± 11.3	1.1		1.1
CClZn	32.6 ± 1.8·32.6 ± 1.8·10.9 ± 0.6	1.3	4.2	1.0	16.9 ± 1.2·16.9 ± 1.2·5.6 ± 0.4	0.4	4.9	7.7
GClZn	19.3 ± 1.8·19.3 ± 1.8·6.4 ± 0.6	0.7	9.5	1.7	26.7 ± 2.8·26.7 ± 2.8·8.9 ± 0.9	0.4	6.7	4.9
GCClZn	17.8 ± 0.8·17.8 ± 0.8·17.8 ± 0.8·5.9 ± 0.3	0.9	3.3	1.8	15.7 ± 0.8·15.7 ± 0.8·15.7 ± 0.8·5.2 ± 0.3	0.5	3.1	8.3
CDn	30.8 ± 1.6·30.8 ± 1.6	0.8	4.5	1.8	19.7 ± 0.6·19.7 ± 0.6	0.6	4.2	2.7★
GDn	34.6 ± 1.8·34.6 ± 1.8	0.8	5.3	1.6	34.1 ± 1.4·34.1 ± 1.4	0.8	5.2	1.5
GCDn	20.6 ± 0.9·20.6 ± 0.9·20.6 ± 0.9	0.7	2.8	2.6★	9.4 ± 0.2·9.4 ± 0.2·9.4 ± 0.2	0.4	5.2	5.6★
DnZn	71.2 ± 6.6·23.7 ± 2.2	1.4		0.8	55.1 ± 2.5·18.4 ± 0.8	1.1		1.0★
CDnZn	27.8 ± 0.8·27.8 ± 0.8·9.3 ± 0.3	0.7	5.0	1.9	34.9 ± 1.0·34.9 ± 1.0·11.6 ± 0.3	1.1	2.4	1.5
GDnZn	33.4 ± 1.3·33.4 ± 1.3·11.1 ± 0.4	0.8	5.5	1.6	34.1 ± 1.3·34.1 ± 1.3·11.4 ± 0.4	0.9	5.2	1.5

GCDnZn	22.5 ± 1.3·22.5 ± 1.3·22.5 ± 1.3·7.5 ± 0.4	0.8	2.6	2.4★	9.1 ± 0.2·9.1 ± 0.2·9.1 ± 0.2·3.0 ± 0.06	0.4	5.4	5.8★
CIbu	151.9 ± 21.6·76.0 ± 10.8	1.6	0.9	2.0	13.2 ± 0.6·6.6 ± 0.3	0.2	6.2	22.7★
GIbu	44.0 ± 4.0·22.0 ± 2.0	0.4	4.2	6.8	49.1 ± 2.3·24.5 ± 1.1	0.4	3.6	6.1
GCIbu	39.3 ± 1.7·39.3 ± 1.7·19.7 ± 0.9	0.8	1.5	7.6	18.4 ± 0.8·18.4 ± 0.8·9.2 ± 0.4	0.4	2.7	16.3★
IbuZn	>75·50	>0.6		<2.0	>75·50	>0.7		<2.0
CIbuZn	>100·50·33.3	>1.1	1.4	<3.0	24.5 ± 2.1·12.2 ± 1.0·8.1 ± 0.7	0.4	3.4	12.3★
GIbuZn	61.8 ± 6.1·30.9 ± 3.0·20.5 ± 2.0	0.6	3.0	4.9	56.9 ± 3.4·28.4 ± 1.7·18.9 ± 1.1	0.6	3.1	5.3
GCIbuZn	52.1 ± 4.5·52.1 ± 4.5·26.1 ± 2.3·17.4 ± 1.5	1.1	1.1	5.7	23.4 ± 1.4·23.4 ± 1.4·11.7 ± 0.7·7.8 ± 0.5	0.6	2.1	12.8★

B. Efflux pump ATPase inhibition

Regimens	SG				OECM-1			
	IC50 (μg/ml; Zn2+,μM)	CI	EI_1[a]	EI_2[b]	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
CCl	>150·150	>5.2	<0.2	<1.3	>150·150	>2.9	<0.4	<3.3
GCl	111.7 ± 2.7·111.7 ± 2.7	2.0	0.7	1.7	83.1 ± 2.9·83.1 ± 2.9	1.8	0.6	6.0★
GCCl	74.8 ± 1.9·74.8 ± 1.9·74.8 ± 1.9	1.6	0.8	2.6	56.3 ± 2.9·56.3 ± 2.9·56.3 ± 2.9	1.2	0.9	8.8★
ClZn	>150·50	>0.9		<1.3	>150 ± 2.1·50 ± 0.7	>0.4		<3.3
CClZn	37.5 ± 2.2·37.5 ± 2.2·12.5 ± 0.7	1.3	0.9	5.2	44.0 ± 5.1·44.0 ± 5.1·14.6 ± 1.7	0.9	1.3	11.3
GClZn	45.1 ± 2.1·45.1 ± 2.1·15.0 ± 0.7	0.8	1.8	4.3	49.1 ± 2.8·49.1 ± 2.8·16.3 ± 0.9	1.1	1.0	10.1
GCClZn	26.4 ± 1.7·26.4 ± 1.7·26.4 ± 1.7·8.8 ± 0.6	0.6	2.4	7.4	29.6 ± 2.0·29.6 ± 2.0·29.6 ± 2.0·9.9 ± 0.7	0.6	1.8	16.8
CDn	32.8 ± 0.7·32.8 ± 0.7	1.6	1.0	1.6	30.7 ± 1.0·30.7 ± 1.0	0.8	1.9	3.3★
GDn	44.0 ± 1.7·44.0 ± 1.7	1.4	1.8	1.2	40.9 ± 2.0·40.9 ± 2.0	1.2	1.2	2.4★
GCDn	26.6 ± 1.3·26.6 ± 1.3·26.6 ± 1.3	0.9	2.3	1.9	20.2 ± 1.0·20.2 ± 1.0·20.2 ± 1.0	0.6	2.6	4.9★
DnZn	87.4 ± 4.3·29.1 ± 1.4	1.8		0.6	64.2 ± 2.4·21.4 ± 0.8	0.7		1.6★
CDnZn	33.0 ± 2.8·33.0 ± 2.8·11.0 ± 0.9	1.6	1.0	1.6	23.5 ± 1.1·23.5 ± 1.1·7.8 ± 0.4	0.7	2.5	4.3★
GDnZn	52.4 ± 2.1·52.4 ± 2.1·17.5 ± 0.7	1.7	1.5	1.0	37.6 ± 2.2·37.6 ± 2.2·12.5 ± 0.7	1.1	1.4	2.7★
GCDnZn	31.5 ± 1.0·31.5 ± 1.0·31.5 ± 1.0·10.5 ± 0.3	1.1	2.0	1.6	20.2 ± 0.7·20.2 ± 0.7·20.2 ± 0.7·6.7 ± 0.2	0.6	2.6	4.9★
CIbu	22.8 ± 2.1·11.4 ± 1.0	0.8	1.5	13.2	29.7 ± 2.6·14.8 ± 1.3	0.6	2.0	10.1
GIbu	58.3 ± 3.0·29.1 ± 1.5	0.9	1.4	5.2	45.2 ± 1.6·22.6 ± 0.8	1.0	1.1	6.6★
GCIbu	41.6 ± 1.8·41.6 ± 1.8·20.8 ± 0.9	0.8	1.5	7.2	17.3 ± 1.2·17.3 ± 1.2·8.6 ± 0.6	0.4	3.1	17.4★
IbuZn	>75·50	>0.7		<2.0	>75·50	>0.6		<2.0
CIbuZn	41.2 ± 4.3·20.6 ± 2.2·13.7 ± 1.4	1.4	0.8	7.3	10.8 ± 0.4·5.4 ± 0.2·3.6 ± 0.1	0.2	5.4	27.8★
GIbuZn	52.0 ± 2.9·26.0 ± 1.4·17.3 ± 1.0	0.9	1.6	5.8	44.5 ± 1.4·22.3 ± 0.7·14.8 ± 0.5	1.1	1.1	6.7★
GCIbuZn	33.9 ± 3.4·33.9 ± 3.4·17.0 ± 1.7·11.3 ± 1.1	0.7	1.8	8.8	19.8 ± 0.6·19.8 ± 0.6·9.9 ± 0.3·6.6 ± 0.2	0.5	2.7	15.2★

Regimens	A549				DLD-1			
	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
CCl	>150·150	>0.8	<2.2	<2.9	>150·150	>1.6	<1.3	<1.2
GCl	71.5 ± 4.0·71.5 ± 4.0	1.2	0.9	6.1★	50.9 ± 0.5·50.9 ± 0.5	1.1	1.1	3.6★
GCCl	46.1 ± 2.9·46.1 ± 2.9·46.1 ± 2.9	0.7	1.6	9.4★	38.3 ± 0.8·38.3 ± 0.8·38.3 ± 0.8	0.8	1.8	4.8★
ClZn	>150 ± 2.8·50 ± 0.9	0.5		2.9	>150 ± 3.7·50 ± 1.2	>1.0		<1.2
CClZn	79.3 ± 3.6·79.3 ± 3.6·26.4 ± 1.2	0.5	4.1	5.5	31.6 ± 2.3·31.6 ± 2.3·10.5 ± 0.8	0.4	6.3	5.9★

(*continued on next page*)

Table 2 (*continued*)

Regimens	A549				DLD-1			
	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
GClZn	47.5 ± 1.6·47.5 ± 1.6·15.8 ± 0.5	0.9	1.4	9.2	37.2 ± 1.5·37.2 ± 1.5·12.4 ± 0.5	0.9	1.6	5.0★
GCClZn	35.5 ± 1.0·35.5 ± 1.0·35.5 ± 1.0·11.8 ± 0.3	0.6	2.1	12.2	19.7 ± 0.5·19.7 ± 0.5·19.7 ± 0.5·6.6 ± 0.2	0.4	3.5	9.4★
CDn	33.1 ± 1.4·33.1 ± 1.4	0.4	9.8	3.3★	23.8 ± 1.1·23.8 ± 1.1	0.4	8.4	3.8★
GDn	39.9 ± 1.4·39.9 ± 1.4	1.0	1.7	2.7★	40.6 ± 2.6·40.6 ± 2.6	1.1	1.4	2.2★
GCDn	26.8 ± 1.1·26.8 ± 1.1·26.8 ± 1.1	0.6	2.8	4.0★	17.1 ± 1.1·17.1 ± 1.1·17.1 ± 1.1	0.4	4.0	5.3★
DnZn	68.3 ± 2.7·22.8 ± 0.9	0.7		1.6★	68.3 ± 3.1·22.8 ± 1.0	0.8		1.3★
CDnZn	35.5 ± 1.4·35.5 ± 1.4·11.8 ± 0.5	0.5	9.1	3.0★	24.0 ± 0.8·24.0 ± 0.8·8.0 ± 0.3	0.4	8.4	3.8★
GDnZn	43.8 ± 0.9·43.8 ± 0.9·14.6 ± 0.3	1.1	1.5	2.5★	39.6 ± 1.1·39.6 ± 1.1·13.2 ± 0.4	1.2	1.5	2.3★
GCDnZn	30.2 ± 0.4·30.2 ± 0.4·30.2 ± 0.4·10.1 ± 0.1	0.7	2.5	3.6★	18.2 ± 0.5·18.2 ± 0.5·18.2 ± 0.5·6.1 ± 0.2	0.5	3.8	5.0★
CIbu	143.2 ± 60.3·71.6 ± 3.0	0.9	2.3	2.1	41.5 ± 2.8·20.7 ± 1.4	0.3	4.8	7.2
GIbu	66.9 ± 2.8·33.5 ± 1.4	1.2	1.0	4.5	62.8 ± 2.9·31.4 ± 1.4	1.3	0.9	4.8
GCIbu	73.9 ± 2.1·73.9 ± 2.1·36.9 ± 1.0	1.2	1.0	4.1	30.4 ± 1.8·30.4 ± 1.8·15.2 ± 0.9	0.5	2.3	9.9★
IbuZn	>75·50	>0.6		<2.0	>75·50	>0.7		<2.0
CIbuZn	>100·50·33.3	>0.7	3.2	<3.0	33.8 ± 1.9·16.9 ± 0.9·11.2 ± 0.6	0.3	5.9	8.9★
GIbuZn	58.0 ± 2.8·29.0 ± 1.4·19.3 ± 0.8	1.1	1.1	5.2	51.4 ± 1.0·25.7 ± 0.5·17.1 ± 0.3	1.1	1.1	5.8★
GCIbuZn	79.0 ± 1.6·79.0 ± 1.6·39.5 ± 0.8·26.3 ± 0.5	1.4	0.9	3.8	23.3 ± 1.2·23.3 ± 1.2·11.6 ± 0.6·7.8 ± 0.4	0.4	3.0	12.9★

C. Antibacterial effects

Regimens	P.g.				UA159			
	IC50(μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50(μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
CCl	52.3 ± 6.2·52.3 ± 6.2	0.5	2.3	11.3	44.3 ± 3.9·44.3 ± 3.9	2.8	5.7	0.4
GCl	69.5 ± 5.9·69.5 ± 5.9	0.7	1.8	8.5	26 ± 1.5·26 ± 1.5	1.6	17.2	0.7
GCCl	113.3 ± 1.9·113.3 ± 1.9·113.3 ± 1.9	1.4	0.8	5.2	36.1 ± 2.6·36.1 ± 2.6·36.1 ± 2.6	2.5	2.6	0.5
ClZn	191.7·63.9	0.5		3.1	39.5 ± 0.3·13.2 ± 0	2.3		0.4
CClZn	42.8 ± 3.3·42.8 ± 3.3·14.1 ± 1.1	0.4	2.9	13.8	17.9 ± 1.9·17.9 ± 1.9·5.6 ± 0.7	1.1	14.0	0.9
GClZn	101.2 ± 3.9·101.2 ± 3.9·33.4 ± 1.3	1.0	1.2	5.8	37.5 ± 2.4·37.5 ± 2.4·12.4 ± 0.8	2.3	11.9	0.5
GCClZn	51.6 ± 1.2·51.6 ± 1.2·51.6 ± 1.2·17.2 ± 0.4	0.7	1.8	11.4	26.4 ± 3.4·26.4 ± 3.4·26.4 ± 3.4·8.8 ± 1.2	1.8	3.6	0.6
CDn	150.9 ± 5.5·150.9 ± 5.5	2.2	0.8	1.1	22 ± 3.1·22 ± 3.1	0.7	11.4	1.8
GDn	45.8 ± 1.3·45.8 ± 1.3	0.7	2.7	3.5	35.4 ± 6·35.4 ± 6	1.0	12.6	1.1
GCDn	39.6 ± 0.4·39.6 ± 0.4·39.6 ± 0.4	0.7	2.3	4.0	23.1 ± 1·23.1 ± 1·23.1 ± 1	0.8	4.1	1.7
DnZn	>150·50	>1.0		<1.1	57.7 ± 3.6·19.2 ± 1.2	1.5		0.7
CDnZn	>100·100·33	>1.5	<1.2	<1.6	30.0 ± 7.2·30.0 ± 7.2·9.9 ± 2.4	0.9	8.4	1.3
GDnZn	39.8 ± 2.5·39.8 ± 2.5·13.1 ± 0.8	0.6	3.1	4.0	30.9 ± 1.9·30.9 ± 1.9·10.2 ± 0.6	0.9	14.4	1.3
GCDnZn	50.7 ± 3.1·50.7 ± 3.1·50.7 ± 3.1·16.9 ± 1	0.9	1.8	3.2	25.5 ± 3.9·25.5 ± 3.9·25.5 ± 3.9·8.5 ± 1.3	0.9	3.7	1.5
CIbu	50.8 ± 1.5·25.4 ± 0.8	0.7	2.4	3.9	65.3 ± 4.1·32.7 ± 2.1	0.6	3.8	3.1
GIbu	46.7 ± 0.9·23.3 ± 0.4	0.6	2.7	4.3	>50·75	0.9	8.9	1.3
GCIbu	48.9 ± 4.3·48.9 ± 4.3·24.5 ± 2.1	0.8	1.9	4.1	51.5 ± 3.2·51.5 ± 3.2·25.7 ± 1.6	0.8	1.9	3.9

IbuZn	>75·50	0.8		1.3	>75·50	0.8		1.3
CIbuZn	>100·50·33	1.4	1.2	2.0	119.3 ± 3.5·59.7 ± 1.7·39.4 ± 1.1	1.1	2.1	1.7
GIbuZn	49.4 ± 1.5·24.7 ± 0.8·16.3 ± 0.5	0.7	2.5	4.0	68.2 ± 2.7·34.1 ± 2.3·22.5 ± 1.5	0.5	6.5	2.9
GCIbuZn	35.3 ± 0.6·35.3 ± 0.6·17.6 ± 0.3·11.8 ± 0.2	0.6	2.6	5.7	24.7 ± 0.6·24.7 ± 0.6·12.4 ± 0.3·8.2 ± 0.2	0.4	3.9	8.1
Regimens	P.a.				S.a.			
	IC50(μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50(μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
CCl	101 ± 2.8·101 ± 2.8	0.3	6.0	9.9	107.2 ± 5.6·107.2 ± 5.6	0.6	2.2	6.3
GCl	26 ± 1.5·26 ± 1.5	0.2	7.1	38.5	>150·150	0.4	6.7	4.5
GCCl	21.3 ± 0.6·21.3 ± 0.6·21.3 ± 0.6	0.2	7.0	46.9	>100·100·100	0.9	1.3	6.7
ClZn	>150·50	0.2		6.7	>150·50	0.2		4.5
CClZn	>100·100·33	0.3	6.0	10.0	106·106·35	0.6	2.2	6.4
GClZn	23.2 ± 2.7·23.2 ± 2.7·7.6 ± 0.9	0.2	7.9	43.1	>100·100·33	0.3	10.0	6.7
GCClZn	22.7 ± 3.1·22.7 ± 3.1·22.7 ± 3.1·7.6 ± 1	0.2	6.6	44.1	>75·75·75·25	0.7	1.8	9.0
CDn	>150·150	>0.4	<4.0	<6.7	41 ± 0.9·41 ± 0.9	1.3	5.8	0.9
GDn	63.9 ± 7.9·63.9 ± 7.9	0.4	2.9	15.6	37.2 ± 0.3·37.2 ± 0.3	1.1	26.9	1.0
GCDn	63.7 ± 3.4·63.7 ± 3.4·63.7 ± 3.4	0.5	2.3	15.7	28.4 ± 1.5·28.4 ± 1.5·28.4 ± 1.5	1.0	4.7	1.3
DnZn	>150·50	>0.2		<6.7	42.8 ± 1.0·14.2 ± 0.3	1.2		0.8
CDnZn	>100·100·33	>0.3	<6.0	<10.0	40.5 ± 4.0·40.5 ± 4.0·13.4 ± 1.3	1.3	5.9	0.9
GDnZn	40.1 ± 3.4·40.1 ± 3.4·13.2 ± 1.1	0.3	4.6	24.9	42.3 ± 1.7·42.3 ± 1.7·14 ± 0.6	1.2	23.6	0.8
GCDnZn	42.7 ± 1.4·42.7 ± 1.4·42.7 ± 1.4·14.3 ± 0.4	0.3	3.5	23.4	35 ± 4.4·35 ± 4.4·35 ± 4.4·11.7 ± 1.5	1.3	3.8	1.0
CIbu	>150·75	1.0	4.0	1.3	130.5 ± 6.2·65.3 ± 3.1	1.2	1.8	1.5
GIbu	103.2 ± 9.2·51.6 ± 4.6	1.1	1.8	1.9	>150·75	0.9	6.7	1.3
GCIbu	39.8 ± 2.2·39.8 ± 2.2·19.9 ± 1.1	0.5	3.7	5.0	>100·100·50	1.3	1.3	2.0
IbuZn	>75·50	0.8		1.3	>75·50	0.8		1.3
CIbuZn	>100·50·33	0.7	6.0	2.0	>100·50·33	0.9	2.4	2.0
GIbuZn	104.2 ± 0.5·52.1 ± 0.3·34.4 ± 0.2	1.1	1.8	1.9	>100·50·33	0.6	10.0	2.0
GCIbuZn	34.5 ± 3·34.5 ± 3·17.2 ± 1.5·11.5 ± 1	0.4	4.3	5.8	>75·75·37.5·25	1.0	1.8	2.7

★Selective anticancer regimens with IC50 less than those of normal gingival epithelial cells (SG) respectively.
C, curcumin; G, green tea polyphenol; Cl, celecoxib; Dn, diclofenac; Ibu, ibuprofen; Zn, ZnSO4.
CI, combination index.
[a] EI-1 for C,G or GC
[b] EI-2 for NSAIDs.

ammonium molybdate color reagents. A standard curve for Pi was established simultaneously.

The synergistic effect (CI < 1) was calculated using the combination index (CI) equation:[34]

$$CI = \frac{(IC_{50})_1 \text{ in combination}}{(IC_{50})_1 \text{ alone}} + \frac{(IC_{50})_2 \text{ in combination}}{(IC_{50})_2 \text{ alone}} + \frac{(IC_{50})_3 \text{ in combination}}{(IC_{50})_3 \text{ alone}}$$

$$CI < 1, synergism; CI = 1, addition; CI > 1, antagonism$$

Efficacy of anticancer or antibacterial effects was calculated by efficacy index (EI):

$$EI = \frac{(IC_{50})_{alone}}{(IC_{50})_{combination}}$$

$$EI > 1, synergism; EI = 1, addition; EI < 1, antagonism$$

Statistics

Results for each experiment were presented as mean ± SEM. One-way ANOVA followed by a post-hoc t-test was employed to assess differences between the groups. The significance level was set at $P < 0.05$.

Results

The potency (IC50) of anticancer effects, efflux pump ATPase inhibition, and antibacterial effects of individual drugs on cultured cells and pathogens

As presented in Table 1A, the anticancer potency (IC50) of various drugs administered alone was predominantly weak, with the exception of methotrexate (MTX), which demonstrated significant potency, followed by 5-fluorouracil (5FU) and cisplatin (Cis). Selective anticancer agents exhibited lower IC50 values on cancer cells compared to normal gingival epithelial cells (SG). In this context, curcumin (C), whether used alone or in combination with green tea polyphenols (G), displayed weak yet selective anticancer effects. Conversely, most non-steroidal anti-inflammatory drugs (NSAIDs) such as Celecoxib (Cl), Diclofenac (Dn), and Ibuprofen (Ibu), as well as chemotherapeutic agents (Cis, 5FU, and MTX), were identified as non-selective anticancer agents, with the exception of Cl on A549 cells and Cis on OECM-1 cells, which were selective (refer to Table 1A).

Table 1B illustrates the inhibition of efflux pump ATPase (IC50) by drugs administered alone. Phytopolyphenols and NSAIDs (Cl, Dn) were found to be weak inhibitors, while three chemotherapeutic agents exhibited moderate non-selective inhibition of the anti-efflux pump ATPase, which was comparable to their anticancer effects.

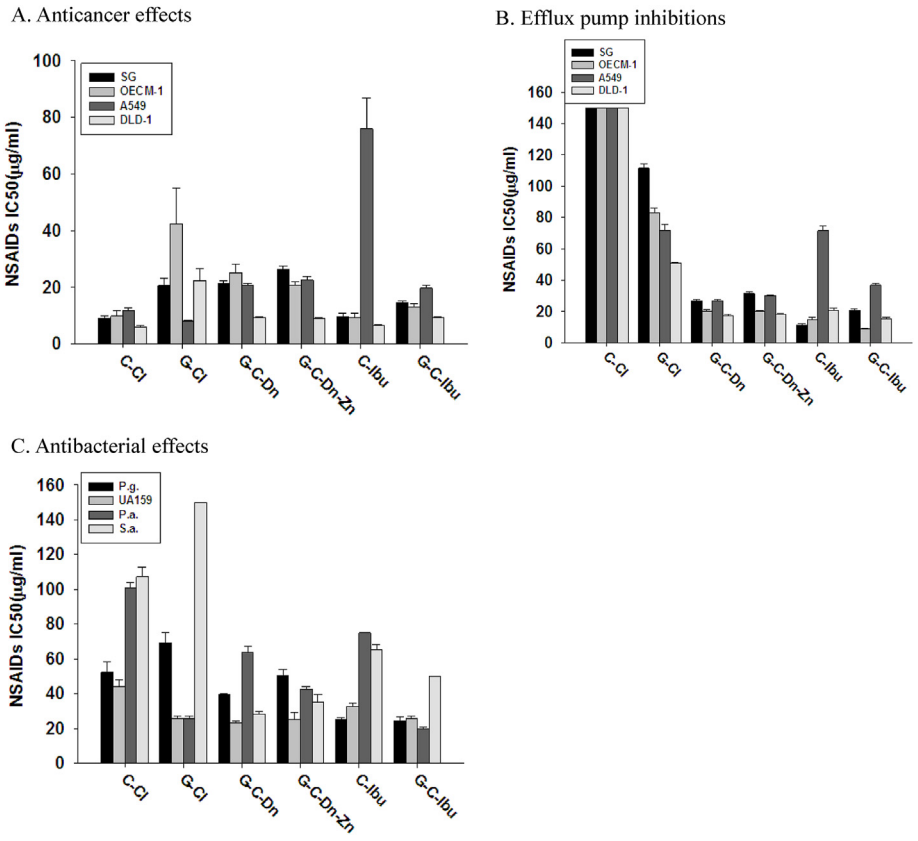

Figure 1 Phytopolyphenols synergistically(CI < 1) enhanced anticancer (A), efflux pump inhibition (B) and antibacterial (C) effects of NSAIDs on cultured cells and pathogens.

Table 1C presents the potency (IC50 in μg/ml; Zn^{2+} in μM) of anti-biofilm formation against four cultured pathogens by drugs used individually. Phytopolyphenols demonstrated moderate inhibitory effects on all four pathogens, whereas Cl exhibited potent activity against UA159, and Dn showed significant effects against both UA159 and S.a. The IC50 values for Cl against UA159 were recorded at 17 ± 1.1 μg/ml, while those for Dn against UA159 and S.a. were 39 ± 1.3 and 35.7 ± 1.5 μg/ml, respectively. The most potent agents, 5FU and MTX, against UA159 exhibited IC50 values of 9.7 ± 0.14 and 5.6 ± 1.3 μg/ml, respectively.

Phytopolyphenols and $ZnSO_4$ enhance the synergistic and selective anticancer (A), anti-efflux pump ATPase (B), and antibacterial (C) effects of NSAIDs on cultured cells and pathogens

As illustrated in Table 2A and Fig. 1A, innovative combinations of NSAIDs with phytopolyphenols and ZnSO4 demonstrated synergistic anticancer effects (combination index (CI) < 1). Specifically, the combinations C·Ibu and G·C·Ibu exhibited selective anticancer activity against OECM-1 and DLD-1 cancer cell lines, while G·C·Dn ± Zn showed selective anticancer effects on both A549 and DLD-1 cells. Notably, G·Cl emerged as a potent selective anticancer agent against A549 lung cancer cells. Furthermore, the anticancer efficacy (EI) were significantly enhanced, particularly within the Ibu regimens, with EI increasing by 11–16 fold in OECM-1, 3–8 fold in A549, and 16–22 fold in DLD-1 cells, respectively. Additionally, these six novel NSAID regimens also demonstrated synergistic selective inhibition of efflux pumps (Table 2B, Fig. 1B) and exhibited antibacterial effects (Table 2C, Fig. 1C).

The regimens NSAIDs-PTM enhanced synergistic and selective anticancer (A), anti-efflux pump ATPase (B), and antibacterial (C) effects of Cis, 5FU, and MTX on cultured cells and pathogens

As illustrated in Table 3A and Figs. 2A, 2D, and 2G, these NSAIDs-PTM regimens significantly improved the anticancer efficacy (Efficacy Index, EI) of Cis, 5FU, and MTX across three different cultured cancer cell lines, with a combination index (CI) of less than one indicating synergy. Notably, the most effective regimens included: (1) G·Cl·Zn·Cis on A549 cells, achieving an EI of 18-fold, and C·Ibu·Zn·Cis on DLD-1 cells, with an EI of 21-fold; (2) C·Dn·Zn·5FU and G·C·Ibu·5FU on DLD-1 cells, both resulting in a 12-fold increase in EI; and (3) C(G, GC)·Cl·Zn·MTX on OECM-1 cells, which increased EI to 8, 4, and 8-fold, respectively, while G·C·Cl·Zn·MTX on A549 cells raised EI to 12-fold, and C·Ibu·Zn·MTX on DLD-1 cells achieved an EI of 15-fold. Furthermore, the majority of these NSAIDs-PTM regimens also exhibited synergistic enhancement (CI < 1) of efflux pump ATPase inhibition and antibacterial efficacy for Cis (Tables 3B and 3C,Figs. 2A, 2B, and 2C), 5FU (Tables 3B and 3C, Figs. 2D, 2E, and 2F), and MTX (Tables 3B and 3C, Figs. 2G, 2H, and 2I).

Table 3 NSAIDs-PTM regimens enhanced synergistic and selective anticancer (A), anti-efflux pump ATPase (B) and antibacterial (C) effects of Cis, 5FU and MTX on cultured cells and pathogens.

Regimens	A. Anticancer effect								
	SG				OECM-1				
	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
ClCis	14.0 ± 0.9·0.47 ± 0.03	0.3	4.2	13.8	69.0 ± 3.7·2.3 ± 1.2	1.2	1.2	2.5
DnCis	21.0 ± 1.6·0.7 ± 0.05	0.6	1.9	9.3	56.6 ± 4.3·1.9 ± 0.14	1.6	0.8	3.1
IbuCis	>75·5	>1.3	<2.0	<1.3	>75·5	>1.4	<2.0	<1.2
Cl5FU	<15·0.5	<0.4	>3.9	>6.2	54.9 ± 3.8·1.8 ± 1.3	1.1	1.5	2.4
Dn5FU	19.8 ± 1.3·0.7 ± 0.04	0.7	2.0	4.5	61.1 ± 6.3·2.0 ± 0.21	1.8	0.7	2.2
Ibu5FU	20.7 ± 1.4·1.4 ± 0.09	0.6	7.2	2.2	>75·5	>1.7	<2.0	<0.9
ClMTX	<15·0.005	<0.5	>3.9	>3.6	<15·0.005	<0.4	>5.6	>4.6
DnMTX	36.7 ± 3.3·0.012 ± 0.001	1.6	1.1	1.5	52.2 ± 3.6·0.017 ± 0.001	1.9	0.9	1.4
IbuMTX	30.8 ± 4.0·0.021 ± 0.003	1.4	4.9	0.9	47.3 ± 2.6·0.032 ± 0.002	1.7	3.2	0.7
CClZnCis	8.9 ± 1.1·8.9 ± 1.1·3.0 ± 0.4·0.296 ± 0.035	0.3	6.5	21.9	11.2 ± 2.0·11.2 ± 2.0·3.7 ± 0.7·0.374 ± 0.066	0.3	7.5	15.5
CDnZnCis	19.1 ± 1.5·19.1 ± 1.5·6.4 ± 0.5·0.7 ± 0.05	0.7	2.1	9.3	25.7 ± 2.5·25.7 ± 2.5·8.6 ± 0.8·0.9 ± 0.08	0.9	1.7	6.4
CIbuZnCis	12.5 ± 1.0·6.2 ± 0.5·4.2 ± 0.3·0.4 ± 0.03	0.2	24.2	16.3	30.1 ± 2.4·15.0 ± 1.2·10.1 ± 0.8·1.0 ± 0.08	0.5	10.0	5.8

(continued on next page)

Table 3 (*continued*)

A. Anticancer effect								
Regimens	SG				OECM-1			
	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
CClZn5FU	7.0 ± 0.6·7.0 ± 0.6·2.3 ± 0.2·0.234 ± 0.02	0.2	8.3	13.3	11.7 ± 3.5·11.7 ± 3.5·3.9 ± 1.2·0.390 ± 0.12	0.3	7.2	11.1
CDnZn5FU	8.7 ± 0.5·8.7 ± 0.5·2.9 ± 0.2·0.3 ± 0.02	0.4	4.6	10.4	20.2 ± 1.6·20.2 ± 1.6·6.8 ± 0.5·0.7 ± 0.05	0.7	2.2	6.2
CIbuZn5FU	5.0 ± 0.2·2.5 ± 0.1·1.7 ± 0.07·0.2 ± 0.007	0.1	60.0	15.5	20.4 ± 1.9·10.2 ± 0.9·6.8 ± 0.6·0.7 ± 0.06	0.4	14.7	6.2
CClZnMTX	9.0 ± 0.3.·9.0 ± 0.3·3.0 ± 0.1·0.003 ± 0.0001	0.4	6.5	6.0	9.3 ± 0.4·9.3 ± 0.4·3.1 ± 0.1·0.003 ± 0.0001	0.3	9.1	7.7
CDnZnMTX	18.1 ± 2.0·18.1 ± 2.0·6.0 ± 0.7·0.006 ± 0.001	0.9	2.2	3.0	24.9 ± 1.6·24.9 ± 1.6·8.3 ± 0.5·0.009 ± 0.001	1.1	1.8	2.6
CIbuZnMTX	9.9 ± 1.1·5.0 ± 0.54·3.3 ± 0.36·0.003 ± 0.0004	0.3	30.0	6.0	22.9 ± 2.8·11.5 ± 1.4·7.6 ± 0.9·0.008 ± 0.0009	0.6	13.0	2.9
GClZnCis	18.5 ± 1.4·18.5 ± 1.4·6.2 ± 0.5·0.62 ± 0.048	0.5	3.1	10.5	36.3 ± 3.8·36.3 ± 3.8·12.1 ± 1.3·1.21 ± 0.13	0.8	2.3	4.8
GDnZnCis	25.1 ± 3.8·25.1 ± 3.8·8.4 ± 1.3·0.8 ± 0.1	0.9	1.6	8.1	32.4 ± 2.9·32.4 ± 2.9·10.8 ± 1.0·1.1 ± 0.1	1.1	1.4	5.3
GIbuZnCis	30.1 ± 2.7·15.1 ± 1.4·10.0 ± 0.9·1.0 ± 0.09	0.5	9.9	6.5	38.4 ± 4.4·19.2 ± 2.2·12.8 ± 1.5·1.3 ± 0.15	0.6	7.8	4.5
GClZn5FU	15.0 ± 0.4·15.0 ± 0.4·5.0 ± 0.1·0.50 ± 0.015	0.5	3.9	6.2	36.9 ± 3.3·36.9 ± 3.3·12.3 ± 1.1·1.23 ± 0.11	0.9	2.3	3.5
GDnZn5FU	21.4 ± 1.7·21.4 ± 1.7·7.1 ± 0.6·0.7 ± 0.06	0.9	1.9	4.5	27.8 ± 2.3·27.8 ± 2.3·9.3 ± 0.8·0.9 ± 0.08	1.0	1.6	4.8
GIbuZn5FU	17.0 ± 1.0·8.5 ± 0.5·5.7 ± 0.3·0.6 ± 0.03	0.4	17.6	5.2	27.8 ± 3.3·13.9 ± 1.7·9.3 ± 1.1·0.9 ± 0.11	0.5	10.8	4.8
GClZnMTX	18.1 ± 0.8·18.1 ± 0.8·6.0 ± 0.3·0.006 ± 0.0003	0.8	3.2	3.0	18.5 ± 1.6·18.5 ± 1.6·6.2 ± 0.5·0.006 ± 0.0005	0.6	4.6	3.8★
GDnZnMTX	34.0 ± 4.2·34.0 ± 4.2·11.3 ± 1.4·0.011 ± 0.001	1.7	1.2	1.6	32.6 ± 1.1·32.6 ± 1.1·10.9 ± 0.4·0.011 ± 0.0004	1.4	1.4	2.1★
GIbuZnMTX	35.3 ± 2.1·17.7 ± 1.0·11.8 ± 0.7·0.012 ± 0.0007	1.0	8.5	1.5	28.7 ± 5.5·14.4 ± 2.7·9.6 ± 1.8·0.010 ± 0.0018	0.7	10.4	2.3★
GCClZnCis	10.5 ± 1.7·10.5 ± 1.7·10.5 ± 1.7·3.5 ± 0.55·0.351 ± 0.056	0.4	5.5	18.5	13.0 ± 4.9·13.0 ± 4.9·13.0 ± 4.9·4.3 ± 1.6·0.434 ± 0.16	0.5	6.5	13.4
GCDnZnCis	31.0 ± 1.9·31.0 ± 1.9·31.0 ± 1.9·10.3 ± 0.6·1.0 ± 0.06	1.3	1.3	6.5	37.8 ± 2.6·37.8 ± 2.6·37.8 ± 2.6·12.6 ± 0.9·1.3 ± 0.09	1.9	1.2	4.5
GCIbuZnCis	27.3 ± 1.4·27.3 ± 1.4·13.7 ± 0.7·9.1 ± 0.5·0.9 ± 0.05	0.5	10.9	7.2	33.3 ± 2.4·33.3 ± 2.4·16.6 ± 1.2·11.1 ± 0.8·1.1 ± 0.08	1.0	9.0	5.3
GCClZn5FU	9.4 ± 0.05·9.4 ± 0.05·9.4 ± 0.05·3.1 ± 0.01·0.312 ± 0.005	0.4	6.2	10.0	14.7 ± 2.7·14.7 ± 2.7·14.7 ± 2.7·4.9 ± 0.9·0.490 ± 0.09	0.6	5.7	8.8
GCDnZn5FU	31.8 ± 3.1·31.8 ± 3.1·31.8 ± 3.1·10.6 ± 1.1·1.1 ± 0.1	1.5	1.3	2.8	39.3 ± 4.6·39.3 ± 4.6·39.3 ± 4.6·13.1 ± 1.5·1.3 ± 0.15	2.0	1.1	3.3
GCIbuZn5FU	11.7 ± 0.9·11.7 ± 0.9·5.9 ± 0.4·3.9 ± 0.3·0.4 ± 0.03	0.3	25.4	7.8	30.9 ± 2.7·30.9 ± 2.7·15.5 ± 1.3·10.3 ± 0.9·1.0 ± 0.09	1.0	9.7	4.3

GCClZnMTX	10.0 ± 0.4·10.0 ± 0.4·10.0 ± 0.4·3.3 ± 0.1·0.003 ± 0.0001	0.5	5.8	6.0	9.1 ± 0.3·9.1 ± 0.3·9.1 ± 0.3·3.0 ± 0.1·0.003 ± 0.0001	0.4	9.3	7.7★
GCDnZnMTX	34.5 ± 2.2·34.5 ± 2.2·34.5 ± 2.2·11.5 ± 0.7·0.011 ± 0.0007	1.9	1.2	1.6	33.7 ± 1.7·33.7 ± 1.7·33.7 ± 1.7·11.2 ± 0.6·0.011 ± 0.0006	1.9	1.3	2.1★
GCIbuZnMTX	20.4 ± 1.0·20.4 ± 1.0·10.2 ± 0.5·6.8 ± 0.3·0.007 ± 0.0003	0.7	14.7	2.6	31.8 ± 2.1·31.8 ± 2.1·15.9 ± 1.1·10.6 ± 0.7·0.011 ± 0.0007	1.3	9.4	2.1
Regimens	A549				DLD-1			
	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
ClCis	14.6 ± 0.7·0.49 ± 0.02	0.5	2.2	20.6★	27.0 ± 0.5·0.90 ± 0.02	0.3	4.8	9.3
DnCis	48.7 ± 0.6·1.6 ± 0.02	1.1	1.1	6.3	53.5 ± 4.4·1.8 ± 0.15	1.2	1.0	4.7
IbuCis	>75·5	>1.0	<2.0	<2.0	>75·5	>1.1	<2.0	<1.7
Cl5FU	<15·0.5	<0.6	>2.1	>7.0	20.2 ± 0.7·0.67 ± 0.02	0.3	6.4	5.4
Dn5FU	45.9 ± 3.0·1.5 ± 0.1	1.3	1.2	2.3	56.8 ± 4.1·1.9 ± 0.14	1.6	0.9	1.9
Ibu5FU	42.0 ± 2.7·2.8 ± 0.18	1.1	3.6	1.3	58.3 ± 4.4·3.9 ± 0.29	1.5	2.6	0.9
ClMTX	18.4·0.006	0.7	1.8	10.2	<15·0.005	<0.2	>8.7	>8.8
DnMTX	59.4 ± 3.9·0.020 ± 0.001	1.4	0.9	3.1	62.5 ± 3.4·0.021 ± 0.001	1.7	0.8	2.1
IbuMTX	69.6 ± 2.5·0.046 ± 0.002	1.2	2.2	1.3	57.4 ± 3.7·0.038 ± 0.002	1.2	2.6	1.2
CClZnCis	18.9 ± 0.2·18.9 ± 0.2·6.3 ± 0.05·0.630 ± 0.005	0.8	1.7	16.0	12.5 ± 1.3·12.5 ± 1.3·4.2 ± 0.5·0.416 ± 0.046	0.3	10.4	20.2
CDnZnCis	40.7 ± 2.7·40.7 ± 2.7·13.6 ± 0.9·1.4 ± 0.09	1.2	1.3	7.2	14.5 ± 1.4·14.5 ± 1.4·4.8 ± 0.5·0.5 ± 0.05	0.5	3.6	16.8★
CIbuZnCis	>75·37.5·25·2.5	>1.1	<4.0	<4.0	11.9 ± 0.5·6.0 ± 0.2·4.0 ± 0.16·0.4 ± 0.02	0.2	25.0	21.0★
CClZn5FU	15.2 ± 1.0·15.2 ± 1.0·5.1 ± 0.4·0.506 ± 0.04	0.7	2.1	6.9	10.9 ± 2.0·10.9 ± 2.0·3.6 ± 0.7·0.364 ± 0.06	0.3	11.9	10.0
CDnZn5FU	12.8 ± 0.5·12.8 ± 0.5·4.3 ± 0.2·0.4 ± 0.02	0.5	4.2	8.8	8.1 ± 0.2·8.1 ± 0.2·2.7 ± 0.1·0.3 ± 0.01	0.3	6.5	12.1★
CIbuZn5FU	18.6 ± 1.4·9.3 ± 0.7·6.2 ± 0.5·0.6 ± 0.05	0.4	16.1	5.9	8.3 ± 0.6·4.2 ± 0.3·2.8 ± 0.2·0.3 ± 0.02	0.2	35.7	12.1
CClZnMTX	21.5 ± 1.0·21.5 ± 1.0·7.2 ± 0.3·0.007 ± 0.0003	1.0	1.5	8.7	15.0 ± 0.4·15.0 ± 0.4·5.0 ± 0.1·0.005 ± 0.0001	0.4	8.7	8.8
CDnZnMTX	30.2 ± 2.8·30.2 ± 2.8·10.1 ± 0.9·0.010 ± 0.001	1.0	1.8	6.1	14.2 ± 0.6·14.2 ± 0.6·4.7 ± 0.2·0.005 ± 0.002	0.6	3.7	8.8★
CIbuZnMTX	57.6 ± 3.0·28.8 ± 1.5·19.2 ± 1.0·0.019 ± 0.001	1.0	5.2	3.2	9.7 ± 0.27·4.8 ± 0.14·3.2 ± 0.09·0.003 ± 0.0001	0.2	31.3	14.7★
GClZnCis	17.0 ± 1.2·17.0 ± 1.2·5.7 ± 0.4·0.57 ± 0.04	0.7	1.9	17.6★	22.9 ± 0.5·22.9 ± 0.5·7.6 ± 0.2·0.76 ± 0.018	0.4	5.7	11.0
GDnZnCis	>75·75·25·2.5	2.1	0.7	4.0	31.7 ± 3.3·31.7 ± 3.3·10.6 ± 1.1·1.1 ± 0.1	0.9	1.7	7.6
GIbuZnCis	39.6 ± 1.3·19.8 ± 1.6·13.2 ± 0.4·1.3 ± 0.04	0.5	7.6	7.8	31.1 ± 2.7·15.5 ± 1.4·10.4 ± 0.9·1.0 ± 0.09	0.4	9.7	8.4★
GClZn5FU	14.9 ± 0.4·14.9 ± 0.4·5.0 ± 0.1·0.50 ± 0.013	0.7	2.2	7.0★	21.0 ± 0.6·21.0 ± 0.6·7.0 ± 0.2·0.70 ± 0.021	0.5	6.2	5.2
GDnZn5FU	37.5 ± 4.2·37.5 ± 4.2·12.5 ± 1.4·1.2 ± 0.14	1.3	1.4	2.9	30.7 ± 2.3·30.7 ± 2.3·10.2 ± 0.8·1.0 ± 0.08	1.1	1.7	3.6
GIbuZn5FU	20.0 ± 1.4·10.0 ± 0.7·6.7 ± 0.5·0.7 ± 0.05	0.4	15.0	5.0	23.5 ± 2.4·11.8 ± 1.2·7.8 ± 0.8·0.8 ± 0.08	0.5	12.7	4.6

(*continued on next page*)

Table 3 (*continued*)

Regimens	A549				DLD-1			
	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
GClZnMTX	15.9 ± 0.8·15.9 ± 0.8·5.3 ± 0.3·0.005 ± 0.0003	0.7	2.0	12.2★	23.3 ± 1.8·23.3 ± 1.8·7.8 ± 0.6·0.008 ± 0.0006	0.5	5.6	5.5
GDnZnMTX	49.9 ± 1.8·49.9 ± 1.8·16.6 ± 0.6·0.017 ± 0.0006	1.5	1.1	3.6	37.0 ± 2.1·37.0 ± 2.1·12.3 ± 0.7·0.012 ± 0.0007	1.2	1.4	3.7
GIbuZnMTX	33.4 ± 1.8·16.7 ± 0.9·11.1 ± 0.6·0.011 ± 0.0006	0.5	9.0	5.5★	34.2 ± 1.8·17.1 ± 0.9·11.4 ± 0.6·0.011 ± 0.0006	0.6	8.8	4.0★
GCClZnCis	16.1 ± 1.8·16.1 ± 1.8·16.1 ± 1.8·5.4 ± 0.6·0.535 ± 0.062	0.8	2.0	18.8	11.8 ± 2.0·11.8 ± 2.0·11.8 ± 2.0·3.9 ± 0.7·0.393 ± 0.068	0.4	11.0	21.4
GCDnZnCis	>60·60·60·20·2	>2.4	<0.9	<5.0	63.7 ± 3.1·63.7 ± 3.1·63.7 ± 3.1·21.2 ± 1.0·2.1 ±0 .01	2.8	0.8	4.0
GCIbuZnCis	23.7 ± 0.5·23.7 ± 0.5·11.8 ± 0.2·7.9 ± 0.2·0.8 ± 0.02	0.6	12.7	12.6★	17.2 ± 0.3·17.2 ± 0.3·8.6 ± 0.2·5.7 ± 0.1·0.6 ± 0.01	0.5	17.4	14.0★
GCClZn5FU	12.5 ± 3.5·12.5 ± 3.5·12.5 ± 3.5·4.2 ± 1.2·0.416 ± 0.12	0.7	2.6	8.4	12.0 ± 1.3·12.0 ± 1.3·12.0 ± 1.3·4.0 ± 0.4·0.400 ± 0.04	0.5	10.8	9.1
GCDnZn5FU	38.4 ± 2.1·38.4 ± 2.1·38.4 ± 2.1·12.8 ± 0.7·1.3 ± 0.07	1.8	1.4	2.7	>60·60·60·20·2	>3.0	<0.9	<1.8
GCIbuZn5FU	13.5 ± 0.5·13.5 ± 0.5·6.7 ± 0.3·4.5 ± 0.2·0.4 ± 0.02	0.4	22.4	8.8	10.2 ± 0.4·10.2 ± 0.4·5.1 ± 0.2·3.4 ± 0.1·0.3 ± 0.01	0.3	29.4	12.1★
GCClZnMTX	14.4 ± 0.6·14.4 ± 0.6·14.4 ± 0.6·4.8 ± 0.2·0.005 ± 0.0002	0.8	2.2	12.2	12.7 ± 0.4·12.7 ± 0.4·12.7 ± 0.4·4.2 ± 0.1·0.004 ± 0.0001	0.5	10.2	11.0
GCDnZnMTX	56.6 ± 5.6·56.6 ± 5.6·56.6 ± 5.6·18.9 ± 1.9·0.019 ± 0.0019	2.4	1.0	3.2	>60·60·60·20·0.02	>2.9	<0.9	<2.2
GCIbuZnMTX	28.7 ± 1.2·28.7 ± 1.2·14.4 ± 0.6·9.6 ± 0.4·0.010 ± 0.0004	0.8	10.4	6.1	14.6 ± 0.5·14.6 ± 0.5·7.3 ± 0.3·4.9 ± 0.2·0.005 ± 0.0002	0.5	20.5	8.8★

B. Efflux pump ATPase inhibition

Regimens	SG				OECM-1			
	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
ClCis	34.4 ± 0.34·1.15 ± 0.01	0.6	5.7	2.3	>150·5	1.8	3.3	0.7
DnCis	39.2 ± 0.8·1.3 ± 0.03	1.3	1.3	2.0	67.7 ± 3.4·2.3 ± 0.11	1.4	1.5	1.5
IbuCis	>75·5	2.4	2.0	0.5	>75·5	2.0	2.0	0.7
Cl5FU	120.4 ± 2.67·4.01 ± 0.09	1.4	1.6	1.3	>150·5	0.8	3.3	2.2
Dn5FU	37.5 ± 1.7·1.3 ± 0.06	1.0	1.4	4.0	68.7 ± 1.6·2.3 ± 0.05	0.9	1.5	4.7
Ibu5FU	27.3 ± 1.5·1.8 ± 0.1	0.5	5.5	2.9	>75·5	1.0	2.0	2.2
ClMTX	38.2 ± 0.47·0.013 ± 0.0002	0.3	5.1	11.5	24.4 ± 0.92·0.008 ± 0.0003	0.1	20.4	73.8★
DnMTX	44.9 ± 2.1·0.015 ± 0.001	1.0	1.2	9.9	68.5 ± 5.7·0.023 ± 0.002	0.7	1.5	25.9

IbuMTX	23.0 ± 2.8·0.015 ± 0.002	0.3	6.5	9.9	>75·0.05	0.6	2.0	11.9
CClZnCis	19.3 ± 0.83·19.3 ± 0.83·6.4 ± 0.28·0.64 ± 0.028	0.9	10.1	4.1	24.8 ± 2.1·24.8 ± 2.1·8.3 ± 0.7·0.83 ± 0.072	0.7	20.1	4.0
CDnZnCis	17.8 ± 1.9·17.8 ± 1.9·5.9 ± 0.6·0.6 ± 0.06	1.1	2.9	4.3	25.1 ± 1.6·25.1 ± 1.6·8.4 ± 0.5·0.8 ± 0.05	0.9	4.0	4.2
CIbuZnCis	20.4 ± 2.7·10.2 ± 1.4·6.8 ± 0.9·0.7 ± 0.09	1.0	14.7	3.7	29.5 ± 2.2·14.7 ± 1.1·9.8 ± 0.7·1.0 ± 0.07	0.9	10.2	3.3
CClZn5FU	17.5 ± 0.64·17.5 ± 0.64·5.8 ± 0.21·0.58 ± 0.021	0.7	11.1	9.0	28.9 ± 3.3·28.9 ± 3.3·9.6 ± 1.1·0.96 ± 0.11	0.7	17.2	11.3
CDnZn5FU	12.1 ± 1.3·12.1 ± 1.3·4.0 ± 0.4·0.4 ± 0.04	0.7	4.3	12.9	21.3 ± 1.2·21.3 ± 1.2·7.1 ± 0.4·0.7 ± 0.04	0.7	4.7	15.4
CIbuZn5FU	13.9 ± 1.3·6.9 ± 0.6·4.6 ± 0.4·0.5 ± 0.04	0.6	21.7	10.4	20.6 ± 1.3·10.3 ± 0.6·6.9 ± 0.4·0.7 ± 0.04	0.5	14.6	15.4
CClZnMTX	23.8 ± 1.2·23.8 ± 1.2·7.9 ± 0.4·0.008 ± 0.0004	0.9	8.2	18.8	27.8 ± 3.7·27.8 ± 3.7·9.3 ± 1.2·0.009 ± 0.0012	0.6	17.9	65.6
CDnZnMTX	21.7 ± 2.0·21.7 ± 2.0·7.2 ± 0.7·0.007 ± 0.0007	1.1	2.4	21.2	26.0 ± 1.2·26.0 ± 1.2·8.7 ± 0.4·0.009 ± 0.0004	0.7	3.8	66.1
CIbuZnMTX	31.4 ± 1.5·15.7 ± 0.7·10.5 ± 0.5·0.010 ± 0.0005	1.1	9.6	14.8	25.0 ± 1.7·12.5 ± 0.9·8.3 ± 0.6·0.008 ± 0.0006	0.5	12.0	74.4★
GClZnCis	27.6 ± 0.8·27.6 ± 0.8·9.2 ± 0.3·0.92 ± 0.03	0.9	7.1	2.8	29.0 ± 0.9·29.0 ± 0.9·9.7 ± 0.3·0.97 ± 0.03	0.9	17.2	3.4
GDnZnCis	36.8 ± 4.5·36.8 ± 4.5·12.3 ± 1.5·1.2 ± 0.15	1.7	1.4	2.2	34.0 ± 3.6·34.0 ± 3.6·11.3 ± 1.2·1.1 ± 0.12	1.4	2.9	3.0★
GIbuZnCis	35.5 ± 1.8·17.7 ± 0.9·11.8 ± 0.6·1.2 ± 0.06	1.1	8.5	2.2	30.8 ± 2.6·15.4 ± 1.3·10.3 ± 0.9·1.0 ± 0.09	1.0	9.7	3.3★
GClZn5FU	23.5 ± 0.6·23.5 ± 0.6·7.8 ± 0.2·0.78 ± 0.02	0.6	8.3	6.7	29.6 ± 1.0·29.6 ± 1.0·9.9 ± 0.3·0.99 ± 0.03	0.8	16.8	10.9
GDnZn5FU	32.1 ± 2.6·32.1 ± 2.6·10.7 ± 0.9·1.1 ± 0.09	1.3	1.6	4.7	35.1 ± 4.5·35.1 ± 4.5·11.7 ± 1.5·1.2 ± 0.15	1.2	2.8	9.0
GIbuZn5FU	22.7 ± 2.4·11.3 ± 1.2·7.6 ± 0.8·0.8 ± 0.08	0.5	13.3	6.5	27.4 ± 2.5·13.7 ± 1.2·9.1 ± 0.8·0.9 ± 0.08	0.7	10.9	12.0
GClZnMTX	36.2 ± 1.2·36.2 ± 1.2·12.1 ± 0.4·0.012 ± 0.0004	0.8	5.4	12.5	35.8 ± 2.5·35.8 ± 2.5·11.9 ± 0.8·0.012 ± 0.0008	0.8	13.9	49.2★
GDnZnMTX	39.0 ± 2.6·39.0 ± 2.6·13.0 ± 0.9·0.013 ± 0.0009	1.4	1.3	11.4	37.2 ± 3.7·37.2 ± 3.7·12.4 ± 1.2·0.012 ± 0.0012	1.2	2.7	49.6★
GIbuZnMTX	34.6 ± 3.4·17.3 ± 1.7·11.5 ± 1.1·0.012 ± 0.0011	0.7	8.7	12.4	28.8 ± 1.4·14.4 ± 0.7·9.6 ± 0.5·0.010 ± 0.005	0.7	10.4	59.5★
GCClZnCis	20.3 ± 0.6·20.3 ± 0.6·20.3 ± 0.6·6.8 ± 0.2·0.68 ± 0.02	0.7	9.6	3.8	27.6 ± 2.2·27.6 ± 2.2·27.6 ± 2.2·9.2 ± 0.7·0.92 ± 0.07	0.9	18.1	3.6
GCDnZnCis	32.3 ± 1.9·32.3 ± 1.9·32.3 ± 1.9·10.8 ± 0.6·1.1 ± 0.06	1.6	1.6	2.4	27.2 ± 1.2·27.2 ± 1.2·27.2 ± 1.2·9.1 ± 0.4·0.9 ± 0.04	1.1	3.7	3.7
GCIbuZnCis	22.9 ± 1.7·22.9 ± 1.7·11.4 ± 0.8·7.6 ± 0.6·0.8 ± 0.06	0.8	13.2	3.3	26.9 ± 1.2·26.9 ± 1.2·13.4 ± 0.6·9.0 ± 0.4·0.9 ± 0.04	0.9	11.2	3.7
GCClZn5FU	14.8 ± 0.4·14.8 ± 0.4·14.8 ± 0.4·4.9 ± 0.2·0.49 ± 0.01	0.4	13.1	10.6	22.0 ± 7.3·22.0 ± 7.3·22.0 ± 7.3·7.3 ± 2.4·0.73 ± 0.24	0.5	22.6	14.8
GCDnZn5FU	29.2 ± 1.6·29.2 ± 1.6·29.2 ± 1.6·9.7 ± 0.5·1.0 ± 0.05	1.3	1.8	5.2	32.0 ± 1.1·32.0 ± 1.1·32.0 ± 1.1·10.7 ± 0.4·1.1 ± 0.04	1.0	3.1	9.8

(*continued on next page*)

Table 3 (*continued*)

B. Efflux pump ATPase inhibition								
Regimens	SG				OECM-1			
	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
GCIbuZn5FU	13.4 ± 0.8·13.4 ± 0.8·6.7 ± 0.4·4.5 ± 0.3·0.4 ± 0.03	0.4	22.4	13.0	23.8 ± 0.7·23.8 ± 0.7·11.9 ± 0.4·7.9 ± 0.2·0.8 ± 0.02	0.6	12.6	13.5
GCClZnMTX	20.2 ± 1.0·20.2 ± 1.0·20.2 ± 1.0·6.7 ± 0.3·0.007 ± 0.0003	0.5	9.6	21.4	25.2 ± 2.7·25.2 ± 2.7·25.2 ± 2.7·8.4 ± 0.9·0.008 ± 0.0009	0.6	19.8	73.8
GCDnZnMTX	34.9 ± 1.1·34.9 ± 1.1·34.9 ± 1.1·11.6 ± 0.4·0.012 ± 0.0004	1.4	1.5	12.4	32.1 ± 1.6·32.1 ± 1.6·32.1 ± 1.6·10.7 ± 0.5·0.011 ± 0.0005	1.0	3.1	54.1★
GCIbuZnMTX	21.9 ± 1.3·21.9 ± 1.3·10.9 ± 0.7·7.3 ± 0.4·0.007 ± 0.0004	0.5	13.8	21.2	32.9 ± 0.9·32.9 ± 0.9·16.4 ± 0.4·11.0 ± 0.3·0.011 ± 0.0003	0.8	9.1	54.1
Regimens	A549				DLD-1			
	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50 (μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
ClCis	111.6 ± 1.69·3.72 ± 0.06	1.1	3.9	1.2	16.7 ± 0.34·0.56 ± 0.01	0.2	11.1	7.0★
DnCis	51.8 ± 0.4·1.7 ± 0.01	0.9	2.1	2.7	57.9 ± 4.1·1.9 ± 0.14	1.1	1.6	2.0
IbuCis	>75·5	1.6	2.0	0.9	>75·5	1.8	2.0	0.8
Cl5FU	126.8 ± 3.69·4.23 ± 0.12	1.3	3.4	0.9	123.2 ± 12.2·4.11 ± 0.41	1.6	1.5	1.1★
Dn5FU	38.4 ± 1.1·1.3 ± 0.04	0.7	2.8	3.1★	57.0 ± 5.4·1.9 ± 0.18	1.1	1.6	2.3
Ibu5FU	46.3 ± 1.3·3.1 ± 0.09	1.1	3.2	1.3	>75·5	1.6	2.0	0.9
ClMTX	69.6 ± 0.98·0.023 ± 0.0003	0.3	6.2	5.7	48.7 ± 1.3·0.016 ± 0.0004	0.4	3.8	10.6
DnMTX	73.9 ± 4.4·0.025 ± 0.001	0.9	1.5	5.2	67.2 ± 8.3·0.022 ± 0.003	0.9	1.3	7.7
IbuMTX	>75·0.05	0.9	2.0	2.6	50.2 ± 2.1·0.033 ± 0.001	0.5	3.0	5.2
CClZnCis	73.7 ± 8.9·73.7 ± 8.9·24.6 ± 3.0·2.46 ± 0.30	1.0	5.9	1.9	25.9 ± 0.84·25.9 ± 0.84·8.6 ± 0.28·0.86 ± 0.028	0.5	7.2	4.5
CDnZnCis	69.9 ± 1.6·69.9 ± 1.6·23.3 ± 0.5·2.3 ± 0.05	1.4	1.5	2.0	24.8 ± 0.9·24.8 ± 0.9·8.3 ± 0.3·0.8 ± 0.03	0.6	3.6	4.9
CIbuZnCis	>75·37.5·25·2.5	1.1	4.0	1.8	25.2 ± 0.9·12.6 ± 0.5·8.4 ± 0.3·0.8 ± 0.03	0.4	11.9	4.9
CClZn5FU	50.5 ± 5.8·50.5 ± 5.8·16.8 ± 1.9·1.68 ± 0.19	0.7	8.6	2.4	22.6 ± 1.2·22.6 ± 1.2·7.5 ± 0.39·0.75 ± 0.039	0.4	8.2	5.9
CDnZn5FU	23.9 ± 0.9·23.9 ± 0.9·8.0 ± 0.3·0.8 ± 0.03	0.5	4.5	5.0	16.0 ± 0.5·16.0 ± 0.5·5.3 ± 0.2·0.5 ± 0.02	0.4	5.6	8.8★
CIbuZn5FU	24.9 ± 0.9·12.5 ± 0.5·8.3 ± 0.3·0.8 ± 0.03	0.4	12.0	5.0	13.7 ± 0.6·6.9 ± 0.3·4.6 ± 0.2·0.5 ± 0.02	0.2	21.7	8.8★
CClZnMTX	70.8 ± 4.7·70.8 ± 4.7·23.6 ± 1.6·0.024 ± 0.0016	0.6	6.1	5.4	30.7 ± 0.8·30.7 ± 0.8·10.2 ± 0.3·0.01 ± 0.0003	0.4	6.0	17.0
CDnZnMTX	50.3 ± 0.5·50.3 ± 0.5·16.8 ± 0.2·0.017 ± 0.0002	0.8	2.2	7.6	27.1 ± 1.1·27.1 ± 1.1·9.0 ± 0.4·0.009 ± 0.0004	0.5	3.3	18.9
CIbuZnMTX	>75·37.5·25·0.025	0.7	4.0	5.2	28.2 ± 1.9·14.1 ± 1.0·9.4 ± 0.6·0.009 ± 0.0006	0.3	10.6	18.9★
GClZnCis	24.7 ± 0.3·24.7 ± 0.3·8.2 ± 0.1·0.82 ± 0.01	0.6	17.6	5.6★	26.0 ± 0.5·26.0 ± 0.5·8.7 ± 0.2·0.87 ± 0.02	0.8	7.1	4.5★
GDnZnCis	69.1 ± 8.9·69.1 ± 8.9·23.0 ± 3.0·2.3 ± 0.30	2.2	1.6	2.0	32.8 ± 2.3·32.8 ± 2.3·10.9 ± 0.8·1.1 ± 0.08	1.2	2.7	3.5★

GIbuZnCis	45.5 ± 1.4·22.8 ± 0.7·15.2 ± 0.5·1.5 ± 0.05	1.2	6.6	3.1	28.4 ± 0.4·14.2 ± 0.2·9.5 ± 0.1·0.9 ± 0.01	0.8	10.6	4.3★
GClZn5FU	21.9 ± 0.5·21.9 ± 0.5·7.3 ± 0.2·0.73 ± 0.02	0.6	19.8	5.5★	20.8 ± 0.4·20.8 ± 0.4·6.9 ± 0.1·0.69 ± 0.01	0.6	8.9	6.4★
GDnZn5FU	44.3 ± 1.9·44.3 ± 1.9·14.8 ± 0.6·1.5 ± 0.06	1.5	2.4	2.7	31.4 ± 1.4·31.4 ± 1.4·10.5 ± 0.5·1.0 ± 0.05	1.1	2.9	4.4★
GIbuZn5FU	20.3 ± 0.3·10.1 ± 0.1·6.8 ± 0.1·0.7 ± 0.01	0.6	14.9	5.7★	22.3 ± 0.8·11.1 ± 0.4·7.4 ± 0.3·0.7 ± 0.03	0.6	13.5	6.3★
GClZnMTX	44.6 ± 3.7·44.6 ± 3.7·14.9 ± 1.2·0.015 ± 0.0012	0.9	9.7	8.7	29.1 ± 0.8·29.1 ± 0.8·9.7 ± 0.3·0.010 ± 0.0003	0.7	6.4	17.0★
GDnZnMTX	65.1 ± 1.3·65.1 ± 1.3·21.7 ± 0.4·0.022 ± 0.0004	1.8	1.7	5.9	40.4 ± 2.1·40.4 ± 2.1·13.5 ± 0.7·0.013 ± 0.0007	1.3	2.2	13.1★
GIbuZnMTX	41.8 ± 1.0·20.9 ± 0.5·13.9 ± 0.3·0.014 ± 0.0003	0.9	7.2	9.3	32.3 ± 1.3·16.1 ± 0.6·10.8 ± 0.4·0.011 ± 0.0004	0.8	9.3	15.5★
GCClZnCis	44.3 ± 7.6·44.3 ± 7.6·44.3 ± 7.6·14.8 ± 2.5·1.48 ± 0.25	1.0	9.8	3.1	19.1 ± 2.1·19.1 ± 2.1·19.1 ± 2.1·6.4 ± 0.7·0.64 ± 0.07	0.6	9.7	6.1★
GCDnZnCis	46.3 ± 1.0·46.3 ± 1.0·46.3 ± 1.0·15.4 ± 0.3·1.5 ± 0.03	1.4	2.3	3.0	57.5 ± 2.4·57.5 ± 2.4·57.5 ± 2.4·19.2 ± 0.8·1.9 ± 0.08	2.0	1.6	2.0
GCIbuZnCis	28.1 ± 0.5·28.1 ± 0.5·14.1 ± 0.3·9.4 ± 0.2·0.9 ± 0.02	0.7	10.6	5.1	23.6 ± 0.5·23.6 ± 0.5·11.8 ± 0.2·7.9 ± 0.2·0.8 ± 0.02	0.7	12.7	4.9★
GCClZn5FU	32.9 ± 21.9·32.9 ± 21.9·32.9 ± 21.9·11.0 ± 7.3·1.10 ± 0.73	0.8	13.2	3.6	15.3 ± 4.2·15.3 ± 4.2·15.3 ± 4.2·5.1 ± 1.4·0.51 ± 0.14	0.4	12.1	8.6★
GCDnZn5FU	46.8 ± 1.9·46.8 ± 1.9·46.8 ± 1.9·15.6 ± 0.6·1.6 ± 0.06	1.5	2.3	2.5	>60·60·60·20·2	>2.1	<1.5	<2.2
GCIbuZn5FU	19.7 ± 0.7·19.7 ± 0.7·9.8 ± 0.4·6.6 ± 0.2·0.7 ± 0.02	0.5	15.3	5.7	13.4 ± 0.2·13.4 ± 0.2·6.7 ± 0.1·4.5 ± 0.1·0.4 ± 0.01	0.3	22.4	11.0★
GCClZnMTX	40.2 ± 4.2·40.2 ± 4.2·40.2 ± 4.2·13.4 ± 1.4·0.013 ± 0.0014	0.8	10.8	10.0	23.0 ± 0.8·23.0 ± 0.8·23.0 ± 0.8·7.7 ± 0.3·0.008 ± 0.0003	0.5	8.1	21.3
GCDnZnMTX	56.1 ± 1.7·56.1 ± 1.7·56.1 ± 1.7·18.7 ± 0.6·0.019 ± 0.0006	1.5	1.9	6.8	>60·60·60·20·0.02	>1.7	<1.5	<8.5
GCIbuZnMTX	31.2 ± 0.9·31.2 ± 0.9·15.6 ± 0.4·10.4 ± 0.3·0.010 ± 0.0003	0.6	9.6	13.0	26.8 ± 0.5·26.8 ± 0.5·13.4 ± 0.2·8.9 ± 0.2·0.009 ± 0.0002	0.6	11.2	18.9

C. Antibacterial effects

Regimens	P.g.				UA159			
	IC50(μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50(μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
ClCis	>150·5	>0.4	<3.9	<9.0	32 ± 1.7·1.1 ± 0.1	1.9	0.5	90.9
DnCis	>150·5	>1.1	<1.1	<9.0	43.3 ± 2.6·1.4 ± 0.1	1.1	0.9	71.4
IbuCis	>75·5	>0.9	<1.3	<9.0	>75·5	>0.8	<1.3	<20.0
Cl5FU	>150·5	>0.3	<3.9	<568.2	30 ± 0.6·1 ± 0.02	1.9	0.6	9.7
Dn5FU	>150·5	>0.9	<1.1	<568.2	34.9 ± 0.4·1.2 ± 0.01	1.0	1.1	8.1
Ibu5FU	>75·5	>0.8	<1.3	<568.2	29.9 ± 0.7·2 ± 0.05	0.5	3.3	4.9
ClMTX	52.9 ± 2.5·17.6 ± 0.9	0.7	11.1	1.7	3.7 ± 0.2·0.001 ± 0.0001	0.2	4.6	5600.0
DnMTX	67 ± 1.3·22.3 ± 0.4	1.1	2.4	1.4	2.5 ± 0.2·0.001 ± 0.0001	0.1	15.6	5600.0

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Table 3 (*continued*)

Regimens	C. Antibacterial effects							
	P.g.				UA159			
	IC50(μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50(μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
IbuMTX	28.7 ± 0.6·19.1 ± 0.4	0.9	3.5	1.6	1.2 ± 0.2·0.001 ± 0.0002	0.01	83.3	5600.0
CClZnCis	61.1 ± 5.1·61.1 ± 5.1·20.4 ± 1.7·2.1 ± 0.2	0.7	9.6	21.4	34.2 ± 1.6·34.2 ± 1.6·11.4 ± 0.5·1.1 ± 0.1	2.2	0.5	90.9
CDnZnCis	72.3 ± 3.7·72.3 ± 3.7·24.1 ± 1.2·2.4 ± 0.1	1.1	2.2	18.7	15.3 ± 1.7·15.3 ± 1.7·5.1 ± 0.6·0.5 ± 0.1	0.5	2.5	200.0
CIbuZnCis	92.9 ± 4.3·46.5 ± 2.2·31 ± 1.4·3.1 ± 0.1	1.4	2.2	14.5	44.3 ± 1.3·22.2 ± 0.6·14.8 ± 0.4·1.5 ± 0.04	0.4	4.5	66.7
CClZn5FU	38.9 ± 4.5·38.9 ± 4.5·13 ± 1.5·1.3 ± 0.2	0.4	15.1	2185.2	28.7 ± 2.4·28.7 ± 2.4·9.6 ± 0.8·1 ± 0.1	1.9	0.6	9.7
CDnZn5FU	>75·75·25·2.5	>1.1	<2.1	<1136.3	14.3 ± 1.3·14.3 ± 1.3·4.8 ± 0.4·0.5 ± 0.04	0.5	2.7	19.4
CIbuZn5FU	134.6 ± 15.6·67.3 ± 7.8·44.9 ± 5.2·4.5 ± 0.5	1.9	1.5	631.3	31.9 ± 0.3·15.9 ± 0.1·10.6 ± 0.1·1.1 ± 0.01	0.4	6.3	8.8
CClZnMTX	39 ± 3.4·39 ± 3.4·13 ± 1.1·13 ± 1.1	0.8	15.1	2.4	2.5 ± 0.4·2.5 ± 0.4·0.9 ± 0.2·0.001 ± 0.02	0.2	6.8	5600.0
CDnZnMTX	69.4 ± 0.4·69.4 ± 0.4·23.1 ± 0.1·23.1 ± 0.1	1.8	2.3	1.3	1.8 ± 0.1·1.8 ± 0.1·0.6 ± 0.03·0.001 ± 0.0001	0.1	21.7	5600.0
CIbuZnMTX	31 ± 0.4·15.5 ± 0.2·10.3 ± 0.1·10.3 ± 0.1	0.8	6.5	3.0	3.8 ± 0.2·1.9 ± 0.1·1.3 ± 0.1·0.001 ± 0.01	0.04	52.6	5600.0
GClZnCis	80.3 ± 2.6·80.3 ± 2.6·26.8 ± 0.9·2.7 ± 0.1	0.9	7.3	16.6	28.8 ± 2.8·28.8 ± 2.8·9.6 ± 0.9·1 ± 0.1	1.8	0.6	100.0
GDnZnCis	44.2 ± 1.6·44.2 ± 1.6·14.7 ± 0.5·1.5 ± 0.1	0.7	3.6	29.9	25.2 ± 1.8·25.2 ± 1.8·8.4 ± 0.6·0.8 ± 0.1	0.7	1.5	125.0
GIbuZnCis	69.5 ± 0.3·34.7 ± 0.1·23.2 ± 0.1·2.3 ± 0.01	1.0	2.9	19.5	49.1 ± 3.2·24.6 ± 1.6·16.4 ± 1.1·1.6 ± 0.1	0.4	4.1	62.5
GClZn5FU	>75·75·25·2.5	>0.8	<7.9	<1136.3	31.1 ± 0.7·31.1 ± 0.7·10.4 ± 0.2·1 ± 0.02	2.0	0.5	9.7
GDnZn5FU	79.7 ± 2.6·79.7 ± 2.6·26.6 ± 0.9·2.7 ± 0.1	1.2	2.0	1052.1	14.6 ± 1.7·14.6 ± 1.7·4.9 ± 0.6·0.5 ± 0.1	0.5	2.7	19.4
GIbuZn5FU	54.2 ± 1.1·27.1 ± 0.5·18.1 ± 0.4·1.8 ± 0.04	0.7	3.7	1578.2	42.7 ± 1.2·21.3 ± 0.6·14.2 ± 0.4·1.4 ± 0.04	0.5	4.7	6.9
GClZnMTX	42.8 ± 3·42.8 ± 3·14.3 ± 1·14.3 ± 1	0.9	13.8	2.1	2.8 ± 0.6·2.8 ± 0.6·0.9 ± 0.2·0.001 ± 0.02	0.2	6.1	5600.0
GDnZnMTX	57.4 ± 2.5·57.4 ± 2.5·19.1 ± 0.8·19.1 ± 0.8	1.5	2.8	1.6	2.5 ± 0.2·2.5 ± 0.2·0.8 ± 0.1·0.001 ± 0.01	0.1	15.6	5600.0
GIbuZnMTX	40.4 ± 2.4·20.2 ± 1.2·13.5 ± 0.8·13.5 ± 0.8	1.0	5.0	2.3	1.5 ± 0.1·0.7 ± 0.05·0.5 ± 0.03·0.001 ± 0.0001	0.01	142.9	5600.0
GCClZnCis	>60·60·60·20·2	>0.8	<9.8	<22.5	23.6 ± 2.3·23.6 ± 2.3·23.6 ± 2.3·7.9 ± 0.8·0.8 ± 0.1	1.6	0.7	125.0
GCDnZnCis	78 ± 3.8·78 ± 3.8·78 ± 3.8·26 ± 1.3·2.6 ± 0.1	1.4	2.0	17.3	15.4 ± 2.9·15.4 ± 2.9·15.4 ± 2.9·5.1 ± 1·0.5 ± 0.1	0.6	2.5	200.0
GCIbuZnCis	45.4 ± 1.6·45.4 ± 1.6·22.7 ± 0.8·15.1 ± 0.5·1.5 ± 0.1	0.8	4.4	29.9	53.6 ± 2.4·53.6 ± 2.4·26.8 ± 1.2·17.9 ± 0.8·1.8 ± 0.1	0.9	3.7	55.6
GCClZn5FU	>60·60·60·20·2	>0.8	<9.8	<1420.4	21.6 ± 2·21.6 ± 2·21.6 ± 2·7.2 ± 0.7·0.7 ± 0.1	1.6	0.8	13.9
GCDnZn5FU	103.7 ± 7.3·103.7 ± 7.3·103.7 ± 7.3·34.6 ± 2.4·3.5 ± 0.2	1.8	1.5	811.7	26.1 ± 2.3·26.1 ± 2.3·26.1 ± 2.3·8.7 ± 0.9·0.9 ± 0.1	1.0	1.5	10.8
GCIbuZn5FU	40.5 ± 4.8·40.5 ± 4.8·20.3 ± 2.4·13.5 ± 1.6·1.4 ± 0.2	0.7	4.9	2029.1	49.6 ± 3·49.6 ± 3·24.8 ± 1.5·16.5 ± 1·1.7 ± 0.1	1.0	4.0	5.7

GCClZnMTX	34.3 ± 3.4·34.3 ± 3.4·34.3 ± 3.4·11.4 ± 1.1·11.4 ± 1.1	0.8	17.2	2.7	2.1 ± 0.3·2.1 ± 0.3·2.1 ± 0.3·0.7 ± 0.1·0.001 ± 0.0001	0.1	8.1	5600.0
GCDnZnMTX	39.6 ± 2.8·39.6 ± 2.8·39.6 ± 2.8·13.2 ± 0.9·13.2 ± 0.9	1.1	4.0	2.3	*4.2 ± 0·4.2 ± 0·4.2 ± 0·1.4 ± 0·0.001 ± 0	0.2	9.3	5600.0
GCIbuZnMTX	31.3 ± 1.2·31.3 ± 1.2·15.6 ± 0.6·10.4 ± 0.4·10.4 ± 0.4	0.9	6.4	3.0	1.8 ± 0.1·1.8 ± 0.1·0.9 ± 0.1·0.6 ± 0.03·0.001 ± 0.0001	0.03	111.1	5600.0

Regimens	P.a.				S.a.			
	IC50(μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50(μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
ClCis	>150·5	>0.4	<6.7	<3.5	>150·5	>0.3	<4.5	<20.0
DnCis	>150·5	>0.4	<6.7	<3.5	45.4 ± 2.1·1.5 ± 0.1	1.3	0.8	66.7
IbuCis	>75·5	>1.0.	<1.3	<3.5	>75·5	>0.8	<1.3	<20.0
Cl5FU	>150·5	>0.3	<6.7	<9.8	15 ± 1·0.5 ± 0.03	0.02	44.9	446.0
Dn5FU	>150·5	>0.3	<6.7	<9.8	8.7 ± 0.6·0.3 ± 0.02	0.2	4.1	743.3
Ibu5FU	>75·5	>0.9	<1.3	<9.8	4.4 ± 0.5·0.3 ± 0.03	0.05	22.7	743.3
ClMTX	>150·50	>0.7	<6.7	<2.0	76.4 ± 5.8·25.5 ± 1.9	0.4	8.8	3.9
DnMTX	>150·50	>0.7	<6.7	<2.0	43.1 ± 0.7·14.4 ± 0.2	1.4	0.8	6.9
IbuMTX	>75·50	>1.3	<1.3	<2.0	>75·50	>1.3	<1.3	<2.0
CClZnCis	91.6 ± 1.8·91.6 ± 1.8·30.5 ± 0.6·3.1 ± 0.1	0.4	10.9	5.6	>75·75·25·2.5	>0.5	<9.0	<40.0
CDnZnCis	>75·75·25·2.5	>0.4	<13.3	<7.0	20.9 ± 0.7·20.9 ± 0.7·7 ± 0.2·0.7 ± 0.02	0.7	1.7	142.9
CIbuZnCis	>75·37.5·25·2.5	>0.7	<2.7	<7.0	>75·37.5·25·2.5	>0.7	<2.7	<40.0
CClZn5FU	87.4 ± 3.2·87.4 ± 3.2·29.1 ± 1.1·2.9 ± 0.1	0.3	11.4	16.9	20.2 ± 1.8·20.2 ± 1.8·6.7 ± 0.6·0.7 ± 0.1	0.1	33.3	318.6
CDnZn5FU	>75·75·25·2.5	>0.3	<13.3	<19.6	15.5 ± 0.3·15.5 ± 0.3·5.2 ± 0.1·0.5 ± 0.01	0.5	2.3	446.0
CIbuZn5FU	>75·37.5·25·2.5	>0.6	<2.7	<19.6	>75·37.5·25·2.5	>0.7	<2.7	<89.2
CClZnMTX	>75·75·25·25	>0.5	<13.3	<4.0	95.4 ± 3·95.4 ± 3·31.8 ± 1·31.8 ± 1	0.9	7.1	3.1
CDnZnMTX	>75·75·25·25	>0.5	<13.3	<4.0	18.8 ± 1.9·18.8 ± 1.9·6.3 ± 0.6·6.3 ± 0.6	0.7	1.9	15.9
CIbuZnMTX	>75·37.5·25·25	>0.8	<2.7	<4.0	>75·37.5·25·25	>1.0	<2.7	<4.0
GClZnCis	23.2 ± 2.7·23.2 ± 2.7·7.7 ± 0.9·0.8 ± 0.1	0.2	43.1	21.8	>75·75·25·2.5	>0.2	<9.0	<40.0
GDnZnCis	42.8 ± 0.8·42.8 ± 0.8·14.3 ± 0.3·1.4 ± 0.03	0.4	23.4	12.4	38.7 ± 0.4·38.7 ± 0.4·12.9 ± 0.1·1.3 ± 0.01	1.1	0.9	76.9
GIbuZnCis	39.6 ± 4.9·19.8 ± 2.4·13.2 ± 1.6·1.3 ± 0.2	0.5	5.1	13.4	>75·37.5·25·2.5	>0.5	<2.7	<40.0
GClZn5FU	20.7 ± 1.9·20.7 ± 1.9·6.9 ± 0.6·0.7 ± 0.1	0.1	48.3	70.0	35.8 ± 3.6·35.8 ± 3.6·11.9 ± 1.2·1.2 ± 0.1	0.1	18.8	185.8
GDnZn5FU	23.2 ± 1.7·23.2 ± 1.7·7.7 ± 0.6·0.8 ± 0.1	0.2	43.1	61.3	27.9 ± 2.5·27.9 ± 2.5·9.3 ± 0.8·0.9 ± 0.1	0.8	1.3	247.8
GIbuZn5FU	23.1 ± 1.6·11.5 ± 0.8·7.7 ± 0.5·0.8 ± 0.1	0.3	8.7	61.3	>75·37.5·25·2.5	>0.5	<2.7	<89.2
GClZnMTX	22.5 ± 2.8·22.5 ± 2.8·7.5 ± 0.9·7.5 ± 0.9	0.2	44.4	13.3	>75·75·25·25	>0.4	<9.0	<4.0
GDnZnMTX	60 ± 1.5·60 ± 1.5·20 ± 0.5·20 ± 0.5	0.6	16.7	5.0	36.2 ± 3.3·36.2 ± 3.3·12.1 ± 1.1·12.1 ± 1.1	1.2	1.0	8.3
GIbuZnMTX	>75·37.5·25·25	>1.0	<2.7	<4.0	>75·37.5·25·25	>0.7	<2.7	<4.0

(*continued on next page*)

Table 3 (*continued*)

Regimens	P.a.				S.a.			
	IC50(μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]	IC50(μg/ml; Zn^{2+},μM)	CI	EI_1[a]	EI_2[b]
GCClZnCis	20.8 ± 1.4·20.8 ± 1.4·20.8 ± 1.4·6.9 ± 0.5·0.7 ± 0.05	0.2	48.1	24.9	>60·60·60·20·2	>0.6	<11.2	<50.0
GCDnZnCis	>60·60·60·20·2	>0.6	<16.7	<8.7	24.5 ± 2.4·24.5 ± 2.4·24.5 ± 2.4·8.2 ± 0.8·0.8 ± 0.1	0.9	1.5	125.0
GCIbuZnCis	15.8 ± 2.4·15.8 ± 2.4·7.9 ± 1.2·5.3 ± 0.8·0.5 ± 0.1	0.2	12.7	34.8	>60·60·30·20·2	>0.8	<3.3	<50.0
GCClZn5FU	20.3 ± 0.8·20.3 ± 0.8·20.3 ± 0.8·6.8 ± 0.3·0.7 ± 0.03	0.2	49.3	70.0	35.5 ± 0.1·35.5 ± 0.1·35.5 ± 0.1·11.8 ± 0.03·1.2 ± 0.003	0.3	19.0	185.8
GCDnZn5FU	46.2 ± 2.1·46.2 ± 2.1·46.2 ± 2.1·15.4 ± 0.7·1.5 ± 0.1	0.4	21.6	32.7	18.9 ± 1.7·18.9 ± 1.7·18.9 ± 1.7·6.3 ± 0.6·0.6 ± 0.1	0.7	1.9	371.7
GCIbuZn5FU	20.5 ± 2.5·20.5 ± 2.5·10.2 ± 1.3·6.8 ± 0.8·0.7 ± 0.1	0.3	9.8	70.0	39.5 ± 2.5·39.5 ± 2.5·19.8 ± 1.2·13.2 ± 0.8·1.3 ± 0.1	0.5	5.1	171.5
GCClZnMTX	17.6 ± 1.9·17.6 ± 1.9·17.6 ± 1.9·5.9 ± 0.7·5.9 ± 0.7	0.2	56.8	16.9	>60·60·60·20·20	>0.8	<11.2	<5.0
GCDnZnMTX	>60·60·60·20·20	>0.7	<16.7	<5.0	25.9 ± 3·25.9 ± 3·25.9 ± 3·8.6 ± 1·8.6 ± 1	1.0	1.4	11.6
GCIbuZnMTX	29.7 ± 3.4·29.7 ± 3.4·14.8 ± 1.7·9.9 ± 1.1·9.9 ± 1.1	0.4	6.8	10.1	>60·60·30·20·20	>1.0	<3.3	<5.0

★Selective anticancer regimens with IC50 less than those of normal gingival epithelial cells (SG) respectively.
Cis, Cisplatin; 5FU,5-Fluorouracil; MTX, Methotrexate.
CI, combination index.
[a] EI-1 for NSAIDs.
[b] EI-2 for Cis, 5FU or MTX.

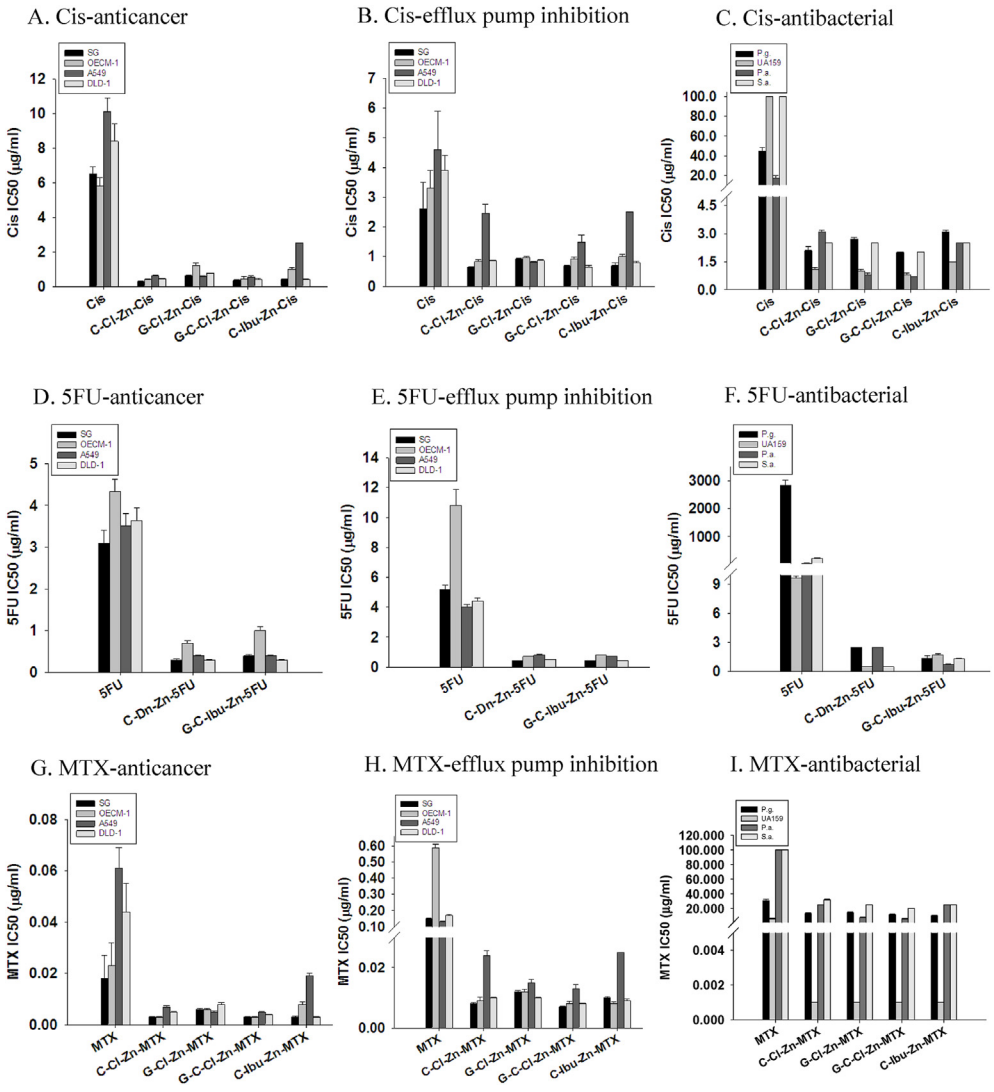

Figure 2 NSAIDs-PTM regimens synergistic (CI < 1) enhancing anticancer efficacy (A,D,G), efflux pump inhibitions (B,E and H) and antibacterial effects (C,F and I) of Cis, 5FU and MTX on cultured cells and pathogens.

Discussion

The global incidence of cancer is on the rise, with notable increases in lung and colon cancers, as well as oral cancers in East Asia, particularly in regions such as Taiwan and India. There is an urgent need for the investigation of effective and safe treatment regimens for cancer chemotherapy. In the present study, the NSAIDs-PTM regimens demonstrated not only synergistic effects (CI < 1) but also selective anticancer properties (IC50 on cancer cells < IC50 on normal SG cells) across three cultured cancer cell lines. Significantly, these regimens were particularly effective in enhancing the efficacy and selectivity of traditionally non-selective anticancer agents, such as Cisplatin (Cis), 5-Fluorouracil (5FU), and Methotrexate (MTX). Furthermore, the NSAIDs-PTM regimens exhibited synergistic inhibition of efflux pump ATPase activity and antibacterial effects, especially when combined with Cis, 5FU, or MTX, indicating their potential to address the prevalent issue of drug resistance.[35–37]

Our laboratory has concentrated on investigating the pleiotropic pharmacological effects of phytopolyphenols (such as curcumin and tea polyphenols),[21–26] which include anti-inflammatory, antioxidant, anticancer, antibacterial, neuroprotective, and immunomodulatory properties. Combinations of these phytopolyphenols with repurposed drugs (including Cl, Dn, Ibu, Cis, 5FU, and MTX) have exhibited synergistic anticancer and antibacterial effects, likely through their antagonistic actions against common risk factors associated with cancer and infectious diseases, such as oxidative stress, inflammation, immunodeficiency, and dysbiosis. The novel PTM regimens have been recognized as innovative and creative approaches, as evidenced by five patents granted for their use in the prevention and management of cancers,[27–29] dementia,[30] and chronic pain.[31] Given that all components of the PTM regimens have demonstrated clinical efficacy and safety, further preclinical and clinical investigations are warranted.

In conclusion, the key findings of this study are as follows: (1) phytopolyphenols significantly enhance the

synergistic selective anticancer efficacy of NSAIDs; (2) NSAIDs-PTM regimens substantially improve the synergistic selective anticancer efficacy of Cis, 5FU, and MTX; and (3) these novel regimens synergistically inhibit both efflux pump activity and pathogenic biofilm formation, with the potential to overcome drug resistance and mitigate the severe side effects associated with cancer chemotherapy. Further clinical studies are encouraged to validate these findings.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgements

The authors greatly appreciate research assistants Miss Chih-Lung Lee and Miss Yi-An Chen for their excellent experiment technique.

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