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Original Article

# Novel regimens of phytopolyphenols and celecoxib enhancing efficacy and selectivity of anticancer effects of chemotherapeutic agents on cultured cancer cells

**Ming-Yung Chou** [a], **Shoei-Yn Lin-Shiau** [a,b*]

[a] *School of Dentistry, College of Oral Medicine, Chung Shan Medical University, Taichung, Taiwan*
[b] *Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan*

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Anti-efflux pump

**Abstract** *Background/purpose:* Explorations of novel regimens enhancing efficacy and selectivity of chemotherapeutic agents are urgent to solve the problems of cancer therapy. This study aimed to explore synergistic anticancer effects of novel regimens of phytopolyphenols [curcumin (C), tea polyphenols (G) or GC] with celecoxib (Cl) and $ZnSO_4$.
*Materials and methods:* Antiproliferative effects of drugs on cultured cancer cells and pathogenic biofilms were assayed by MTT and optical density (OD600) respectively; their inhibition on efflux pump ($Na^+$-$K^+$-ATPase) was measured by colorimetric methods. Synergistic (CI $<$ 1) anticancer effects were evaluated by the equations of combination index (CI) and efficacy index (EI).
*Results:* Both Cl and methotrexate (MTX) alone exhibited inhibitory effects not only on proliferation and efflux pump of cultured cancer cells but also pathogenic biofilm formation. Phytopolyphenols (P) and MTX potentiated these inhibitory effects of Cl. In addition, novel regimens containing Cl, memantine (Mem) or thioridazine (TRZ) further enhanced not only efficacy and selectivity of anticancer effects but also inhibition on efflux pump and pathogenic biofilm formation of four chemotherapeutic agents (MTX, cisplatin, 5-fluorouracil and doxorubicin) respectively.
*Conclusion:* In this study, novel regimens of phytopolyphenols (P), targeting drugs (T; Cl, Mem or TRZ) and metal ions (M; $ZnSO_4$) so called PTM regimens exerted not only by themselves but also markedly potentiated efficacy and selectivity of anticancer effects of four chemotherapeutic agents. Because of their potent inhibitions on efflux pump and pathogenic biofilm formation, these combinatorial novel regimens were expected to be able to overcome the problems of multidrug resistant cancers and merit for further clinical studies.

* Corresponding author. School of Dentistry, College of Oral Medicine, Chung Shan Medical University, No. 110, Section 1, Jian-Guo N. Rd., Taichung 40201, Taiwan.
*E-mail address:* syshiau@csmu.edu.tw (S.-Y. Lin-Shiau).

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## Introduction

Celecoxib is a non-steroidal anti-inflammatory drug and also a selective COX-2 inhibitor.[1–3] It exerts a variety of therapeutic effects such as anticancer, antidiabetes, an inhibitor of cytokine (IL-1β, TNFα) production and treatment of osteoarthritis and rheumatoid arthritis. Recently, a combination of celecoxib (Cl) and methotrexate (MTX) for the therapy of oral cancers, so called metronomic therapy appeared very promising.[4–6] It is recognized as promising, effective, safe and economic clinical regimens for oral cancers especially for the recurrent oral cancers.[7–9]

In our laboratory, we are exploring the pharmaceutical compositions of phytopolyphenols (P), targeting drug (T) and metal ion (M), so called PTM regimens for preventions and managements of chronic diseases (cancers, infectious diseases, diabetes, dementia, chronic pain, etc.), which have been approved as innovative and creative by five patents.[10] It is conceivable to evaluate whether PTM regimens containing either Cl or/and MTX having synergistic anticancer and antibacterial effects.

In this study, PTM regimens containing various targeting drugs (Cl,memantine (Mem),thioridazine (TRZ)) were evaluated for their anticancer, efflux pump inhibition and antibacterial effects on three cultured cancer cells (oral, lung, and colon cancers) and four cultured pathogens. In addition, the influence of these regimens on the efficacy and selectivity of anticancer effects of four chemotherapeutic agents (methotrexate, cisplatin, 5-fluorouracil and doxorubicin) were evaluated. The results obtained indicated that almost all of these combinatorial PTM regimens increased efficacy and selectivity of anticancer effects of these four chemotherapeutic agents. Because of their effective, safe and economic properties, these regimens merit for further clinical studies.

## Materials and methods

### Drugs studied

Curcumin(C) was purchased from Merck Co. (Darmstadt, German). Green tea polyphenols purified from *Camellia sinensis* containing 98% polyphenols (75% catechins HPLC and 50% EGCG HPLC) similar to polyphenol E[11] was purchased from Hunan Huacheng Biotech, Inc. China. Cisplatin (Cis), memantine (Mem) and thioridazine (TRZ) were from Sigma Chemical Company (St. Louis, Missouri, USA). Celecoxib, methotrexate (MTX),5-fluorouracil (5-FU) and doxorubicin (Dx) were from Department of Pharmacy, Chung-Shan Medical University Hospital (Taichung, Taiwan).

### Anticancer and antibacterial potencies ($IC_{50}$)

Antiproliferative effects of cultured cells (SG, normal gingival epithelium; OECM-1, oral squamous cell carcinoma; A549, lung cancer; DLD-1, colon cancer) and pathogens (P.g., *Porphyromonas gingivalis*; UA159, *Streptococcus mutans*; P. a., *Pseudomonas aeruginosa*; S. a., *Staphylococcus aureus*) were assayed by MTT test and optical density (OD600) respectively.[12] $IC_{50}$ [concentration (μg/ml, Zn concentation is μM) for 50% inhibition] was calculated from the concentration–inhibition curve. Efflux pump ($Na^+$-$K^+$-ATPase) activity was assayed by colorimetric method as described previously.[13]

Synergistic effect (CI < 1) was calculated from equation of combination index (CI):[14]

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

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

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

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

### Statistics

Results for each experiment were represented as mean ± SEM. One way ANOVA followed by a post-hoc t test was used to evaluate differences between the groups. The level of significance was defined as $P < 0.05$.

## Results

As shown on Tables 1A and 1B, both celecoxib (Cl) and methotrexate (MTX) alone inhibited not only growth but also efflux pump of three cultured cancer cells (oral OECM-1; lung A549; colon DLD-1 cancer cells). Curcumin (C), tea polyphenol (G) or GC potentiated as well as MTX on these two inhibitory effects of Cl (Fig. 1A and B). The novel PTM regimens of phytopolyphenols (P; C or G or GC) and targeting drugs (T; Cl) and metal ion (M; $ZnSO_4$) exhibited synergistic (CI < 1) anticancer effects on three cultured cancer cells (Table 1A), but those regimens containing MTX instead of Cl had no such synergistic effects (Tables 2A and 2B). On the other hand, PTM regimens containing Cl showed mostly synergistic (CI < 1) inhibitory effects on the biofilm formation of four cultured pathogens (Table 1C, Fig. 1C).

**Table 1** Inhibitory potency ($IC_{50}$), synergism (CI < 1) and altered efficacy (EI) of celecoxib (Cl) in various regimens on proliferation (A) and efflux pump (B) of four cultured cells.

A. Proliferation

| Drugs | SG | | | OECM-1 | | |
|---|---|---|---|---|---|---|
| | $IC_{50}$(μg/ml) | CI | EI | $IC_{50}$ (μg/ml) | CI | EI |
| Cl | 58.2 | | | 84.5 | | |
| ClZn | 32.8•10.9 | 0.6 | 1.8 | >150•50 | >1.9 | 0.6 |
| CCl | 9.1•9.1 | 0.2 | 6.4 | 9.8•9.8 | 0.2 | 8.6 |
| CClZn | 14.0•14.0•4.6 | 0.3 | 4.2 | 19.5•19.5•6.5 | 0.3 | 4.3 |
| GCl | 20.7•20.7 | 0.5 | 2.8 | 42.5•42.5 | 0.7 | 2.0 |
| GClZn | 18.8•18.8•6.3 | 0.5 | 3.1 | 36.6•36.6•12.2 | 0.6 | 2.3 |
| GCCl | 14.3•14.3•14.3 | 0.4 | 4.1 | 21.1•21.1•21.1 | 0.7 | 4.0 |
| GCClZn | 11.7•11.7•11.7•3.9 | 0.3 | 5.0 | 20.5•20.5•20.5•6.8 | 0.7 | 4.1 |
| Drugs | A549 | | | DLD-1 | | |
| | $IC_{50}$ (μg/ml) | CI | EI | $IC_{50}$ (μg/ml) | CI | EI |
| Cl | 32.2 | | | 129.8 | | |
| ClZn | 27.0•9.0 | 0.9 | 1.2 | 120.9•40.3 | 1.1 | 1.1 |
| CCl | 11.7•11.7 | 0.4 | 2.8 | 6.0•6.0 | 0.1 | 21.6 |
| CClZn | 51.2•51.2•17.1 | 2.0 | 0.6 | 23.8•23.8•7.9 | 0.5 | 5.5 |
| GCl | 8.0•8.0 | 0.3 | 4.0 | 22.3•22.3 | 0.3 | 5.8 |
| GClZn | 19.3•19.3•6.4 | 0.7 | 1.7 | 26.7•26.7•8.9 | 0.4 | 4.9 |
| GCCl | 14.4•14.4•14.4 | 0.7 | 2.2 | 17.9•17.9•17.9 | 0.5 | 7.3 |
| GCClZn | 18.6•18.6•18.6•6.2 | 0.9 | 1.7 | 17.8•17.8•17.8•5.9 | 0.5 | 7.3 |

B. Efflux pump

| Drugs | SG | | | OECM-1 | | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ (μg/ml) | CI | EI | $IC_{50}$ (μg/ml) | CI | EI |
| Cl | 418.7 | | | 522.8 | | |
| ClZn | >150•50 | >0.5 | 2.8 | >150•50 | >0.4 | 3.5 |
| CCl | >150•150 | >4.8 | 2.8 | >150•150 | >2.8 | 3.5 |
| CClZn | 40.8•40.8•13.6 | 1.3 | 10.3 | 46.9•46.9•15.6 | 0.9 | 11.1 |
| GCl | 111.7•111.7 | 1.6 | 3.7 | 83.1•83.1 | 1.8 | 6.3 |
| GClZn | 45.1•45.1•15.0 | 0.7 | 9.3 | 49.1•49.1•16.3 | 1.1 | 10.6 |
| GCCl | 74.8•74.8•74.8 | 1.4 | 5.6 | 56.3•56.3•56.3 | 1.2 | 9.3 |
| GCClZn | 28.2•28.2•28.2•9.4 | 0.5 | 14.8 | 28.2•28.2•28.2•9.4 | 0.6 | 18.5 |
| Drugs | A549 | | | DLD-1 | | |
| | $IC_{50}$ (μg/ml) | CI | EI | $IC_{50}$ (μg/ml) | CI | EI |
| Cl | >1000 | | | 383.9 | | |
| ClZn | >150•50 | >0.3 | 6.7 | >150•50 | >0.6 | 2.6 |
| CCl | >150•150 | >0.6 | 6.7 | >150•150 | >1.1 | 2.6 |
| CClZn | 116.5•116.5•38.8 | 0.6 | 8.6 | 56.7•56.7•18.9 | 0.5 | 6.8 |
| GCl | 71.5•71.5 | 1.2 | 14.0 | 50.9•50.9 | 1.0 | 7.5 |
| GClZn | 47.5•47.5•15.8 | 0.8 | 21.1 | 37.2•37.2•12.4 | 0.8 | 10.3 |
| GCCl | 46.1•46.1•46.1 | 0.7 | 21.7 | 38.3•38.3•38.3 | 0.7 | 10.0 |
| GCClZn | 34.0•34.0•34.0•11.3 | 0.5 | 29.4 | 20.7•20.7•20.7•6.9 | 0.4 | 18.5 |

C. Biofilm of pathogens

| Drugs | P.g. | | | UA159 | | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ (μg/ml) | CI | EI | $IC_{50}$ (μg/ml) | CI | EI |
| Cl | 589.3 ± 18.7 | | | 17.3 ± 2 | | |
| ClZn | 191.7•63.9 | 0.5 | 3.1 | 39.5 ± 0.3•13.2 ± 0 | 2.3 | 0.4 |
| CCl | 52.3 ± 6.2•52.3 ± 6.2 | 0.5 | 11.3 | 32.8•32.8 | 2.0 | 0.5 |
| CClZn | 42.8 ± 3.3•42.8 ± 3.3•14.1 ± 1.1 | 0.5 | 13.8 | 17.9 ± 1.9•17.9 ± 1.9•5.6 ± 0.7 | 1.1 | 1.0 |
| GCl | 69.5 ± 5.9•69.5 ± 5.9 | 0.7 | 8.5 | 25.6•25.6 | 1.5 | 0.7 |

**Table 1** (*continued*)

| C. Biofilm of pathogens | | | | | | |
|---|---|---|---|---|---|---|
| Drugs | P.g. | | | UA159 | | |
| | $IC_{50}$ (μg/ml) | CI | EI | $IC_{50}$ (μg/ml) | CI | EI |
| GClZn | 101.2 ± 3.9•101.2 ± 3.9•33.4 ± 1.3 | 1.1 | 5.8 | 37.5 ± 2.4•37.5 ± 2.4•12.4 ± 0.8 | 2.3 | 0.5 |
| GCCl | 118.8•118.8•118.8 | 1.5 | 5.0 | 26•26•26 | 1.8 | 0.7 |
| GCClZn | 51.6 ± 1.2•51.6 ± 1.2•51.6 ± 1.2•17.2 ± 0.4 | 0.7 | 11.4 | 26.4 ± 3.4•26.4 ± 3.4•26.4 ± 3.4•8.8 ± 1.2 | 1.8 | 0.7 |
| Drugs | P.a. | | | S.a. | | |
| | $IC_{50}$ (μg/ml) | CI | EI | $IC_{50}$ (μg/ml) | CI | EI |
| Cl | >1000 | | | 675.8 ± 5 | | |
| ClZn | >150•50 | >0.2 | <6.7 | >150•50 | >0.3 | <4.5 |
| CCl | 102.6•102.6 | 0.3 | 9.7 | 76.5•76.5 | 0.4 | 8.8 |
| CClZn | >100•100•33 | >0.3 | <10.0 | 106•106•35 | 0.6 | 6.4 |
| GCl | 34.2•34.2 | 0.2 | 29.2 | >150•150 | >0.4 | <4.5 |
| GClZn | 23.2 ± 2.7•23.2 ± 2.7•7.6 ± 0.9 | 0.2 | 43.1 | >100•100•33 | >0.3 | <6.8 |
| GCCl | 21.9•21.9•21.9 | 0.2 | 45.7 | >100•100•100 | >0.9 | <6.8 |
| GCClZn | 22.7 ± 3.1•22.7 ± 3.1•22.7 ± 3.1•7.6 ± 1 | 0.2 | 44.1 | >75•75•75•25 | >0.7 | <9.0 |

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*. C, curcumin; G, green tea polyphenol; Zn, $ZnSO_4$. CI, combination index. EI, efficacy index of Cl of anticancer effects (A), efflux pump inhibition (B) and anti-biofilm formation (C).

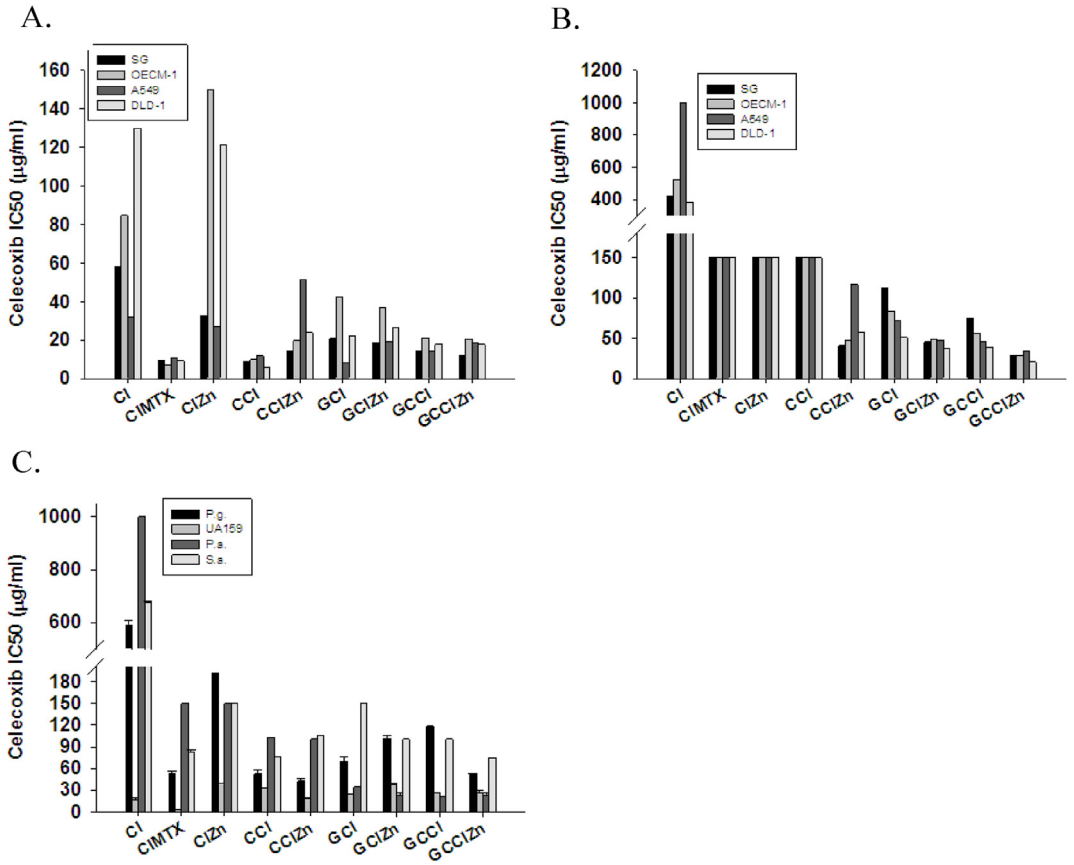


**Figure 1** Celecoxib(Cl) combined with curcumin (C) or tea polyphenols (G) potentiating the inhibitory effects on proliferation(A), efflux pump(B) of cancer cells and pathogenic biofilm formation(C).

**Table 2** Inhibitory potency ($IC_{50}$), synergism (CI < 1) and altered efficacy (EI) of methotraxete (MTX) in various regimens on proliferation (A) and efflux pump (B) of four cultured cells.

| A. Proliferation | | | | | | |
|---|---|---|---|---|---|---|
| Drugs | SG | | | OECM-1 | | |
| | $IC_{50}$ (μg/ml) | CI | EI | $IC_{50}$ (μg/ml) | CI | EI |
| MTX | 0.018 | | | 0.023 | | |
| ClMTX | 9.6•0.032 | 1.9 | 0.6 | 6.8•0.023 | 1.1 | 1.0 |
| MTXZn | 0.03•0.09 | 1.7 | 0.6 | 0.02•0.07 | 0.9 | 1.2 |
| CMTX | 0.82•0.03 | 1.7 | 0.6 | 0.65•0.02 | 0.9 | 1.2 |
| CMTXZn | 0.55•0.02•0.06 | 1.1 | 0.9 | 0.51•0.02•0.05 | 0.9 | 1.2 |
| GCMTX | 0.49•0.49•0.02 | 1.1 | 0.9 | 0.55•0.55•0.02 | 0.9 | 1.2 |
| GCMTXZn | 0.58•0.58•0.02•0.06 | 1.1 | 0.9 | 0.56•0.56•0.02•0.06 | 0.9 | 1.2 |
| Drugs | A549 | | | DLD-1 | | |
| | $IC_{50}$ (μg/ml) | CI | EI | $IC_{50}$ (μg/ml) | CI | EI |
| MTX | 0.061 | | | 0.044 | | |
| ClMTX | 10.7•0.036 | 0.9 | 1.7 | 9.2•0.031 | 0.8 | 1.4 |
| MTXZn | 0.05•0.15 | 0.8 | 1.2 | 0.06•0.19 | 1.4 | 0.7 |
| CMTX | 1.25•0.04 | 0.7 | 1.5 | 1.01•0.03 | 0.7 | 1.5 |
| CMTXZn | 2.65•0.09•0.27 | 1.5 | 0.7 | 3.06•0.10•0.31 | 2.3 | 0.4 |
| GCMTX | 1.69•1.69•0.06 | 1.0 | 1.0 | 2.66•2.66•0.09 | 2.1 | 0.5 |
| GCMTXZn | 1.8•1.8•0.06•0.18 | 1.0 | 1.0 | 3.3•3.3•0.11•0.33 | 2.6 | 0.4 |
| B. Efflux pump | | | | | | |
| Drugs | SG | | | OECM-1 | | |
| | $IC_{50}$ (μg/ml) | CI | EI | $IC_{50}$ (μg/ml) | CI | EI |
| MTX | 0.15 | | | 0.59 | | |
| ClMTX | >150•0.5 | >3.7 | 0.3 | >150•0.5 | >1.1 | 1.2 |
| MTXZn | 0.06•0.17 | 0.4 | 2.5 | 0.08•0.25 | 0.1 | 7.4 |
| CMTX | 1.43•0.05 | 0.4 | 3.0 | 2.09•0.07 | 0.2 | 8.5 |
| CMTXZn | 0.98•0.03•0.10 | 0.2 | 4.9 | 1.17•0.04•0.12 | 0.1 | 14.9 |
| GCMTX | 1.15•1.15•0.04 | 0.3 | 3.7 | 1.42•1.42•0.05 | 0.1 | 11.9 |
| GCMTXZn | 0.88•0.88•0.03•0.09 | 0.2 | 4.9 | 0.87•0.87•0.03•0.09 | 0.1 | 19.8 |
| Drugs | A549 | | | DLD-1 | | |
| | $IC_{50}$ (μg/ml) | CI | EI | $IC_{50}$ (μg/ml) | CI | EI |
| MTX | 0.13 | | | 0.17 | | |
| ClMTX | >150•0.5 | >4.0 | 0.3 | >150•0.5 | >3.3 | 0.3 |
| MTXZn | 0.06•0.19 | 0.5 | 2.2 | 0.07•0.21 | 0.4 | 2.4 |
| CMTX | 1.68•0.06 | 0.5 | 2.2 | 1.84•0.06 | 0.4 | 2.8 |
| CMTXZn | 1.29•0.04•0.13 | 0.3 | 3.3 | 0.95•0.03•0.10 | 0.2 | 5.7 |
| GCMTX | 1.49•1.49•0.05 | 0.4 | 2.6 | 1.61•1.61•0.05 | 0.3 | 3.4 |
| GCMTXZn | 1.21•1.21•0.04•0.12 | 0.3 | 3.3 | 0.92•0.92•0.03•0.09 | 0.2 | 5.7 |

C, curcumin; G, green tea polyphenol; Zn, $ZnSO_4$. EI, efficacy index of MTX of anticancer effects (A) and efflux pump inhibition (B).

As shown on Tables 3—6, influences of various PTM regimens containing three different targeting drugs [Cl or, memantine (Mem) or thioridazine (TRZ)] on anticancer and efflux pump inhibitory effects of four chemotherapeutic agents (MTX, cisplatin (Cis), 5-fluorouracil (5-FU) and doxorubicin (Dx)) showed further profoundly in enhancing efficacy and selectivity of anticancer effects of four chemotherapeutic agents. The best regimens GC-TRZ-Zn on MTX (Table 3A and Fig. 2A), GC-Mem-Zn on Cis and 5-FU (Tables 4A and 5B; Fig. 2B and C) and GC-Cl-Zn on Dx (Table 6B and Fig. 2D) exhibited the most powerful synergistic and selective anticancer effects respectively.

Similarly, these combinatorial PTM regimens also showed enhanced inhibitory effects on efflux pumps of cultured cancer cells by four chemotherapeutic agents respectively (Tables 3B,4B, 5B and 6B; Fig. 3).

As shown on Table 7 and Fig. 4, PTM regimens containing Cl, Mem or TRZ also showed enhancing antibacterial effects of four chemotherapeutic agents. The best synergistic (CI = 0.2—0.5) antibacterial effects of these combinatorial PTM regimens were shown on P.a. biofilm formation (Table 7) and those exhibited extraordinary effective antibacterial effects of MTX on UA159 (a cariogenic pathogen, Fig. 4A).

**Table 3** Altered potency ($IC_{50}$), synergism (CI < 1) and efficacy of methotraxete (MTX) various regimens on proliferation (A) and efflux pump (B) of four cultured cells.

| A. Proliferation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Drugs | SG | | | | OECM-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| MTX | 0.018 | | | | 0.023 | | | |
| CClZnMTX | 4.9•4.9•1.6•0.016 | 1.0 | 11.9 | 1.1 | 3.3•3.3•1.1•0.011 | 0.5 | 25.6 | 2.1 |
| GClZnMTX | 5.9•5.9•2.0•0.020 | 1.3 | 9.9 | 0.9 | 4.8•4.8•1.6•0.016 | 0.8 | 17.6 | 1.4 |
| GCClZnMTX | 5.1•5.1•5.1•1.7•0.017 | 1.1 | 11.4 | 1.1 | 6.0•6.0•6.0•2.0•0.020 | 1.1 | 14.1 | 1.2 |
| CMemZnMTX | 5.7•5.7•1.9•0.019 | 1.2 | 9.7 | 0.9 | 4.6•4.6•1.5•0.015 | 0.7 | 25.1 | 1.5 |
| CTRZZnMTX | 7.3•0.7•2.4•0.024 | 1.5 | 12.0 | 0.8 | 3.3•0.3•1.1•0.011 | 0.5 | 39.1 | 2.1 |
| GMemZnMTX | 6.0•6.0•2.0•0.020 | 1.3 | 9.2 | 0.9 | <7.5•7.5•2.5•0.025 | <1.2 | 15.4 | 0.9 |
| GTRZZnMTX | >7.5•0.75•2.5•0.025 | >1.5 | 11.2 | 0.7 | 2.5•0.3•0.8•0.008 | 0.4 | 39.1 | 2.9 |
| GCMemZnMTX | 6.6•6.6•6.6•2.2•0.022 | 1.4 | 8.4 | 0.8 | 2.2•2.2•2.2•0.7•0.007 | 0.4 | 52.5 | 3.3 |
| GCTRZZnMTX | 8.2•8.2•0.8•2.7•0.027 | 1.7 | 10.5 | 0.7 | 1.0•1.0•0.1•0.3•0.003 | 0.2 | 117.3 | 7.7 |
| Drugs | A549 | | | | DLD-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| MTX | 0.061 | | | | 0.044 | | | |
| CClZnMTX | 8.5•8.5•2.8•0.028 | 0.8 | 3.8 | 2.2 | 6.1•6.1•2.0•0.020 | 0.6 | 21.3 | 2.2 |
| GClZnMTX | 12.4•12.4•4.1•0.041 | 1.1 | 2.6 | 1.5 | 9.2•9.2•3.1•0.031 | 0.8 | 14.1 | 1.4 |
| GCClZnMTX | 9.3•9.3•9.3•3.1•0.031 | 1.0 | 3.5 | 2.0 | 7.8•7.8•7.8•2.6•0.026 | 0.8 | 16.6 | 1.7 |
| CMemZnMTX | 14.9•14.9•5.0•0.050 | 1.1 | 7.3 | 1.2 | 6.7•6.7•2.2•0.022 | 0.7 | 9.2 | 2.0 |
| CTRZZnMTX | 7.4•0.7•2.5•0.025 | 0.5 | 15.2 | 2.4 | 6.4•0.6•2.1•0.021 | 0.6 | 15.3 | 2.1 |
| GMemZnMTX | 23.8•23.8•7.9•0.079 | 1.7 | 4.6 | 0.8 | >7.5•7.5•2.5•0.025 | >0.7 | 8.2 | 1.8 |
| GTRZZnMTX | 8.6•0.9•2.9•0.029 | 0.6 | 11.8 | 2.1 | >7.5•0.75•2.5•0.025 | >0.7 | 12.2 | 1.8 |
| GCMemZnMTX | 8.4•8.4•8.4•2.8•0.028 | 0.7 | 12.9 | 2.2 | 4.0•4.0•4.0•1.3•0.013 | 0.4 | 15.4 | 3.4 |
| GCTRZZnMTX | 5.6•5.6•0.6•1.9•0.019 | 0.5 | 17.7 | 3.2 | 4.1•4.1•0.4•1.4•0.014 | 0.5 | 22.9 | 3.1 |
| B. Efflux pump | | | | | | | | |
| Drugs | SG | | | | OECM-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| MTX | 0.15 | | | | 0.59 | | | |
| CClZnMTX | 22.3•22.3•7.4•0.07 | 1.2 | 18.8 | 2.1 | >75•75•25•0.25 | >1.9 | 7.0 | 2.4 |
| GClZnMTX | 40.9•40.9•13.6•0.14 | 1.6 | 10.2 | 1.1 | 53.1•53.1•17.7•0.18 | 1.5 | 9.8 | 3.3 |
| GCClZnMTX | 13.4•13.4•13.4•4.5•0.045 | 0.6 | 31.2 | 3.3 | 47.1•47.1•47.1•15.7•0.157 | 1.3 | 11.1 | 3.8 |
| CMemZnMTX | 8.9•8.9•3.0•0.30 | 2.4 | 6.9 | 0.5 | 8.8•8.8•2.9•0.029 | 0.3 | 12.0 | 20.5 |
| CTRZZnMTX | 13.0•1.3•4.3•0.043 | 0.9 | 5.4 | 3.4 | 7.8•0.8•2.6•0.026 | 0.3 | 9.4 | 22.9 |
| GCMemZnMTX | 10.5•10.5•10.5•3.5•0.035 | 0.4 | 5.8 | 4.2 | 7.3•7.3•7.3•2.4•0.024 | 0.2 | 14.5 | 24.8 |
| GCTRZZnMTX | 14.5•14.5•1.5•4.8•0.048 | 0.5 | 4.7 | 3.1 | 5.3•5.3•0.5•1.8•0.018 | 0.2 | 15.1 | 33.0 |
| Drugs | A549 | | | | DLD-1 | | | |
| | $IC_{50}$(μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| MTX | 0.13 | | | | 0.17 | | | |
| CClZnMTX | >75•75•25•0.25 | >2.3 | 13.3 | 0.5 | 20.2•20.2•6.7•0.07 | 0.6 | 19.0 | 2.4 |
| GClZnMTX | >75•75•25•0.25 | >3.2 | 13.3 | 0.5 | 18.0•18.0•6.0•0.06 | 0.7 | 21.3 | 2.8 |
| GCClZnMTX | 59.0•59.0•59.0•19.7•0.197 | 2.4 | 16.9 | 0.7 | 15.7•15.7•15.7•5.2•0.052 | 0.6 | 24.4 | 3.3 |
| CMemZnMTX | 11.0•11.0•3.7•0.037 | 0.5 | 7.4 | 3.5 | 9.9•9.9•3.3•0.033 | 0.4 | 5.6 | 5.2 |
| CTRZZnMTX | 14.5•1.5•4.8•0.048 | 0.6 | 6.8 | 2.7 | 9.4•0.9•3.1•0.031 | 0.4 | 8.6 | 5.5 |
| GCMemZnMTX | 12.5•12.5•12.5•4.2•0.042 | 0.3 | 6.5 | 3.1 | 11.1•11.1•11.1•3.7•0.037 | 0.4 | 5.0 | 4.6 |
| GCTRZZnMTX | 14.5•14.5•1.5•4.8•0.048 | 0.4 | 6.8 | 2.7 | 9.1•9.1•0.9•3.0•0.030 | 0.3 | 8.6 | 5.7 |

C, curcumin; G, green tea polyphenol; Cl, celecoxib; Mem, memantine; TRZ, thioridazine; Zn, $ZnSO_4$. (A) EI1 and EI2 are efficacy index of anticancer effects of Cl (Mem or TRZ) and MTX respectively. (B) EI1 and EI2 are efficacy index of efflux pump inhibition of Cl (Mem or TRZ) and MTX respectively.

**Table 4** Altered potency ($IC_{50}$), synergism (CI < 1) and efficacy of cisplatin (Cis) various regimens on proliferation (A) and efflux pump (B) of four cultured cells.

| A. Proliferation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Drugs | SG | | | | OECM-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| Cis | 6.5 | | | | 5.8 | | | |
| CClZnCis | 8.9•8.9•3.0•0.296 | 0.3 | 6.5 | 22.0 | 11.2•11.2•3.7•0.374 | 0.26 | 7.5 | 15.5 |
| GClZnCis | 18.5•18.5•6.2•0.62 | 0.5 | 3.1 | 10.5 | 36.3•36.3•12.1•1.21 | 0.83 | 2.3 | 4.8 |
| GCClZnCis | 10.5•10.5•10.5•3.5•0.351 | 0.4 | 5.5 | 18.5 | 13.0•13.0•13.0•4.3•0.434 | 0.50 | 6.5 | 13.4 |
| CMemZnCis | 27•27•9•0.9 | 0.8 | 2.0 | 7.2 | 21.3•21.3•7.1•0.7 | 0.4 | 5.4 | 8.3 |
| CTRZZnCis | 19.8•2.0•6.6•0.7 | 0.5 | 4.2 | 9.3 | 26.1•2.6•8.7•0.9 | 0.5 | 4.5 | 6.4 |
| GCMemZnCis | 27.3•27.3•27.3•9.1•0.9 | 1.0 | 2.0 | 7.2 | 24.7•24.7•24.7•8.2•0.8 | 0.9 | 4.7 | 7.3 |
| GCTRZZnCis | 23.8•23.8•2.4•8.0•0.8 | 0.7 | 3.5 | 8.1 | 20.9•20.9•2.1•7.0•0.7 | 0.7 | 5.6 | 8.3 |
| Drugs | A549 | | | | DLD-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| Cis | 10.1 | | | | 8.4 | | | |
| CClZnCis | 18.9•18.9•6.3•0.630 | 0.80 | 1.7 | 16.0 | 12.5•12.5•4.2•0.416 | 0.31 | 10.4 | 20.2 |
| GClZnCis | 17.0•17.0•5.7•0.57 | 0.69 | 1.9 | 17.7 | 22.9•22.9•7.6•0.76 | 0.42 | 5.7 | 11.1 |
| GCClZnCis | 16.1•16.1•16.1•5.4•0.535 | 0.84 | 2.0 | 18.9 | 11.8•11.8•11.8•3.9•0.393 | 0.39 | 11.0 | 21.4 |
| CMemZnCis | 30.7•30.7•10.2•1.0 | 0.6 | 3.5 | 10.1 | 13.3•13.3•4.4•0.4 | 0.4 | 4.6 | 21.0 |
| CTRZZnCis | 26.9•2.7•9.0•0.9 | 0.6 | 3.9 | 11.2 | 18.6•1.9•6.2•0.6 | 0.5 | 4.8 | 14.0 |
| GCMemZnCis | 28.3•28.3•28.3•9.4•0.9 | 0.9 | 3.8 | 11.2 | 13•13•13•4.3•0.4 | 0.5 | 4.7 | 21.0 |
| GCTRZZnCis | 32.6•32.6•3.3•10.9•1.1 | 1 | 3.2 | 9.2 | 13.0•13.0•1.3•4.3•0.4 | 0.5 | 7.1 | 21.0 |
| B. Efflux pump | | | | | | | | |
| Drugs | SG | | | | OECM-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| Cis | 2.6 | | | | 3.3 | | | |
| CClZnCis | 19.3•19.3•6.4•0.64 | 0.9 | 21.7 | 4.1 | 24.8•24.8•8.3•0.83 | 0.7 | 21.1 | 4.0 |
| GClZnCis | 27.6•27.6•9.2•0.92 | 0.8 | 15.2 | 2.8 | 29.0•29.0•9.7•0.97 | 0.9 | 18.0 | 3.4 |
| GCClZnCis | 20.3•20.3•20.3•6.8•0.68 | 0.7 | 20.6 | 3.8 | 27.6•27.6•27.6•9.2•0.92 | 0.9 | 18.9 | 3.6 |
| CMemZnCis | 19.0•19.0•6.3•0.6 | 1.1 | 3.2 | 4.3 | 23.0•23.0•7.7•0.8 | 0.9 | 4.6 | 4.1 |
| CTRZZnCis | 18.8•1.9•6.3•0.6 | 1.1 | 3.7 | 4.3 | 17.1•1.7•5.7•0.6 | 0.7 | 4.4 | 5.5 |
| GCMemZnCis | 17.0•17.0•17.0•5.7•0.6 | 0.8 | 3.6 | 4.3 | 6.0•6.0•6.0•2.0•0.2 | 0.2 | 17.6 | 16.5 |
| GCTRZZnCis | 15.2•15.2•1.5•5.1•0.5 | 0.7 | 4.7 | 5.2 | 19.9•19.9•2.0•6.6•0.7 | 0.9 | 3.8 | 4.7 |
| Drugs | A549 | | | | DLD-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| Cis | 4.6 | | | | 3.9 | | | |
| CClZnCis | 73.7•73.7•24.6•2.46 | 0.9 | 13.6 | 1.9 | 25.9•25.9•8.6•0.86 | 0.4 | 14.8 | 4.5 |
| GClZnCis | 4.7•24.7•8.2•0.82 | 0.3 | 40.5 | 5.6 | 26.0•26.0•8.7•0.87 | 0.8 | 14.8 | 4.5 |
| GCClZnCis | 44.3•44.3•44.3•14.8•1.48 | 1.0 | 22.6 | 3.1 | 19.1•19.1•19.1•6.4•0.64 | 0.5 | 20.1 | 6.1 |
| CMemZnCis | 30.0•30.0•10.0•1.0 | 0.7 | 2.7 | 4.6 | 21.0•21.0•7.0•0.7 | 0.7 | 2.6 | 5.6 |
| CTRZZnCis | 27.6•2.8•9.2•0.9 | 0.6 | 3.6 | 5.1 | 29.3•2.9•9.8•1.0 | 0.8 | 2.7 | 3.9 |
| GCMemZnCis | 25.0•25.0•25.0•8.3•0.8 | 0.8 | 3.3 | 5.8 | 22.0•22.0•22.0•7.3•0.7 | 0.9 | 2.5 | 5.6 |
| GCTRZZnCis | 19.3•19.3•1.9•6.4•0.6 | 0.6 | 5.4 | 7.7 | 14.2•14.2•1.4•4.7•0.5 | 0.53 | 5.5 | 8 |

C, curcumin; G, green tea polyphenol; Cl, celecoxib; Mem, memantine; TRZ, thioridazine; Zn, $ZnSO_4$.(A) EI1 and EI2 are efficacy index of anticancer effects of Cl (Mem or TRZ) and Cis respectively. (B) EI1 and EI2 are efficacy index of efflux pump inhibition of Cl (Mem or TRZ) and Cis respectively.

## Discussion

Incidence of oral cancers is high in East Asia such as India and Taiwan.[7] Development of novel therapeutic regimens such as metronomic celecoxib (Cl) and methotrexate (MTX) chemotherapy were proved to be effective, safe and economic.[7–9]

In our laboratory, we have studied the biological effects of curcumin (C) and tea polyphenols (G) for more than 30 years.[15–18] Their pleiotropic pharmacological effects of antioxidant, anti-inflammatory, anticancer, antibacterial, neuroprotective and immunomodulatory effects account for their potentiality in preventions and managements of chronic diseases (cancers, infectious diseases, diabetes,

**Table 5** Altered potency ($IC_{50}$), synergism (CI < 1) and efficacy of 5-fluorouracil (5-FU) various regimens on proliferation (A) and efflux pump (B) of four cultured cells.

| A. Proliferation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Drugs | SG | | | | OECM-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| 5FU | 3.1 | | | | 4.3 | | | |
| CClZn5FU | 7.0•7.0•2.3•0.234 | 0.2 | 8.3 | 13.3 | 11.7•11.7•3.9•0.390 | 0.3 | 7.2 | 11.0 |
| GClZn5FU | 15.0•15.0•5.0•0.50 | 0.5 | 3.9 | 6.2 | 36.9•36.9•12.3•1.23 | 0.9 | 2.3 | 3.5 |
| GCClZn5FU | 9.4•9.4•9.4•3.1•0.312 | 0.4 | 6.2 | 9.9 | 14.7•14.7•14.7•4.9•0.490 | 0.6 | 5.7 | 8.8 |
| CMemZn5FU | 16.6•16.6•5.5•0.6 | 0.6 | 3.3 | 5.2 | 18.7•18.7•6.2•0.6 | 0.4 | 6.2 | 7.2 |
| CTRZZn5FU | 12.1•1.2•4.0•0.4 | 0.3 | 7.0 | 7.8 | 20.7•2.1•6.9•0.7 | 0.5 | 5.6 | 6.1 |
| GCMemZn5FU | 27.5•27.5•27.5•9.2•0.9 | 1.1 | 2.0 | 3.4 | 26.4•26.4•26.4•8.8•0.9 | 1.0 | 4.4 | 4.8 |
| GCTRZZn5FU | 14.3•14.3•1.4•4.8•0.5 | 0.5 | 6.0 | 6.2 | 27.9•27.9•2.8•9.3•0.9 | 1.0 | 4.2 | 4.8 |
| Drugs | A549 | | | | DLD-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| 5FU | 3.5 | | | | 3.6 | | | |
| CClZn5FU | 15.2•15.2•5.1•0.506 | 0.7 | 2.1 | 6.9 | 10.9•10.9•3.6•0.364 | 0.3 | 11.9 | 9.9 |
| GClZn5FU | 14.9•14.9•5.0•0.50 | 0.7 | 2.2 | 7.0 | 21.0•21.0•7.0•0.70 | 0.5 | 6.2 | 5.1 |
| GCClZn5FU | 12.5•12.5•12.5•4.2•0.42 | 0.7 | 2.6 | 8.4 | 12.0•12.0•12.0•4.0•0.4 | 0.5 | 10.8 | 9.0 |
| CMemZn5FU | 17.6•17.6•5.9•0.6 | 0.5 | 6.2 | 5.8 | 7.8•7.8•2.6•0.3 | 0.3 | 7.9 | 12.0 |
| CTRZZn5FU | 17.2•1.7•5.7•0.6 | 0.5 | 6.2 | 5.8 | 7.6•0.8•2.6•0.3 | 0.3 | 11.5 | 12.0 |
| GCMemZn5FU | 26.8•26.8•26.8•8.9•0.9 | 1.0 | 4.0 | 3.9 | 9.6•9.6•9.6•3.2•0.3 | 0.5 | 6.4 | 12.0 |
| GCTRZZn5FU | 20.1•20.1•2.0•6.7•0.7 | 0.7 | 5.3 | 5.0 | 8.5•8.5•0.9•2.8•0.3 | 0.4 | 10.2 | 12.0 |
| B. Efflux pump | | | | | | | | |
| Drugs | SG | | | | OECM-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| 5FU | 5.2 | | | | 10.8 | | | |
| CClZn5FU | 17.5•17.5•5.8•0.58 | 0.7 | 23.9 | 8.9 | 28.9•28.9•9.6•0.96 | 0.7 | 18.1 | 11.3 |
| GClZn5FU | 23.5•23.5•7.8•0.78 | 0.5 | 17.8 | 6.6 | 29.6•29.6•9.9•0.99 | 0.8 | 17.7 | 10.9 |
| GCClZn5FU | 14.8•14.8•14.8•4.9•0.49 | 0.4 | 28.3 | 10.5 | 22.0•22.0•22.0•7.3•0.73 | 0.5 | 23.8 | 14.8 |
| CMemZn5FU | 16.0•16.0•5.3•0.5 | 0.8 | 3.8 | 10.4 | 12.0•12.0•4.0•0.4 | 0.4 | 8.8 | 27.0 |
| CTRZZn5FU | 11.2•1.1•3.7•0.4 | 0.6 | 6.4 | 13.0 | 14.4•1.4•4.8•0.5 | 0.5 | 5.4 | 21.6 |
| GCMemZn5FU | 14.2•14.2•14.2•4.7•0.5 | 0.6 | 4.3 | 10.4 | 14.6•14.6•14.6•4.9•0.5 | 0.5 | 7.2 | 21.6 |
| GCTRZZn5FU | 14.5•14.5•1.5•4.8•0.5 | 0.5 | 4.7 | 10.4 | 27.7•27.7•2.8•9.2•0.9 | 1.0 | 2.7 | 12.0 |
| Drugs | A549 | | | | DLD-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| 5FU | 4.0 | | | | 4.4 | | | |
| CClZn5FU | 50.5•50.5•16.8•1.68 | 0.7 | 19.8 | 2.4 | 22.6•22.6•7.5•0.75 | 0.4 | 17.0 | 5.9 |
| GClZn5FU | 21.9•21.9•7.3•0.73 | 0.6 | 45.7 | 5.5 | 20.8•20.8•6.9•0.69 | 0.6 | 18.5 | 6.4 |
| GCClZn5FU | 32.9•32.9•32.9•11.0•1.10 | 0.8 | 30.4 | 3.7 | 15.3•15.3•15.3•5.1•0.51 | 0.4 | 25.1 | 8.7 |
| CMemZn5FU | 20.0•20.0•6.7•0.7 | 0.5 | 4.1 | 5.7 | 12.8•12.8•4.3•0.4 | 0.4 | 4.3 | 11.0 |
| CTRZZn5FU | 18.7•1.9•6.2•0.6 | 0.4 | 5.4 | 6.7 | 22.0•2.2•7.4•0.8 | 0.6 | 3.5 | 5.5 |
| GCMemZn5FU | 18.4•18.4•18.4•6.1•0.6 | 0.6 | 4.4 | 6.7 | 13.8•13.8•13.8•4.6•0.5 | 0.6 | 4.0 | 8.8 |
| GCTRZZn5FU | 23.6•23.6•2.4•7.8•0.8 | 0.8 | 4.3 | 5.0 | 8.1•8.1•0.8•2.7•0.3 | 0.3 | 9.6 | 14.7 |

C, curcumin; G, green tea polyphenol; Cl, celecoxib; Mem, memantine; TRZ, thioridazine; Zn, $ZnSO_4$. (A) EI1 and EI2 are efficacy index of anticancer effects of Cl (Mem or TRZ) and 5FU respectively. (B) EI1 and EI2 are efficacy index of efflux pump inhibition of Cl (Mem or TRZ) and 5FU respectively.

etc.), especially in combination with the targeting drugs (T; Mem, TRZ, etc.) and metal ions (M; $ZnSO_4$), so called PTM regimens, which produced synergistic and selective anticancer and antibacterial effects.[12,19] They were approved by five patents as innovative and creative regimens.[10] In this study, we showed that PTM regimens containing Cl, Mem or TRZ exhibited synergistic anticancer effects. Moreover, these PTM regimens markedly enhanced both efficacy and selectivity of anticancer effects of four frequently used chemotherapeutic agents (MTX, Cis, 5-FU and Dx).

Recently, increasing incidences of multidrug resistance (MDR) in cancers and infectious diseases become a big challenging problem to be solved.[20,21] Increased efflux

**Table 6** Altered potency ($IC_{50}$), synergism (CI < 1) and efficacy of doxorubicin (Dx) various regimens on proliferation (A) and efflux pump (B) of four cultured cells.

A. Proliferation

| Drugs | SG | | | | OECM-1 | | | |
|---|---|---|---|---|---|---|---|---|
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| Dx | 0.06 | | | | >0.3 | | | |
| CClZnDx | 8.5•8.5•2.8•0.003 | 0.25 | 6.9 | 20.0 | 12.4•12.4•4.1•0.004 | 0.23 | 6.8 | 75.0 |
| GClZnDx | 17.7•17.7•5.9•0.006 | 0.53 | 3.3 | 10.0 | 35.9•35.9•12.0•0.012 | 0.66 | 2.4 | 25.0 |
| GCClZnDx | 6.6•6.6•6.6•2.2•0.002 | 0.22 | 8.8 | 30.0 | 19.5•19.5•19.5•6.5•0.006 | 0.66 | 4.3 | 50.0 |
| CMemZnDx | >25•25•8.3•0.008 | 0.73 | 2.2 | 7.5 | 22.2•22.2•7.4•0.007 | 0.3 | 5.2 | 42.9 |
| CTRZZnDx | >25•2.5•8.3•0.008 | 0.58 | 3.4 | 7.5 | 18.9•1.9•6.3•0.006 | 0.3 | 6.2 | 50.0 |
| GCMemZnDx | >20•20•20•6.7•0.006 | 0.59 | 2.8 | 10.0 | 35.2•35.2•35.2•11.7•0.012 | 1.0 | 3.3 | 25.0 |
| GCTRZZnDx | >20•20•2.0•6.7•0.006 | 0.47 | 4.2 | 10.0 | 19.8•19.8•2.0•6.6•0.007 | 0.6 | 5.9 | 42.9 |
| Drugs | A549 | | | | DLD-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| Dx | 0.14 | | | | 0.15 | | | |
| CClZnDx | 14.8•14.8•4.9•0.005 | 0.61 | 2.2 | 28.0 | 12.8•12.8•4.3•0.004 | 0.30 | 10.1 | 37.5 |
| GClZnDx | 14.0•14.0•4.7•0.005 | 0.56 | 2.3 | 28.0 | 24.1•24.1•8.0•0.008 | 0.40 | 5.4 | 18.8 |
| GCClZnDx | 10.7•10.7•10.7•3.6•0.004 | 0.55 | 3.0 | 35.0 | 10.3•10.3•10.3•3.4•0.003 | 0.32 | 12.6 | 50.0 |
| CMemZnDx | 46.8•46.8•15.6•0.016 | 0.91 | 2.3 | 8.8 | 16.2•16.2•5.4•0.005 | 0.51 | 3.8 | 30.0 |
| CTRZZnDx | 27.4•2.7•9.1•0.009 | 0.53 | 3.9 | 15.6 | 16.8•1.7•5.6•0.006 | 0.45 | 5.4 | 25.0 |
| GCMemZnDx | 37.1•37.1•37.1•12.4•0.012 | 1.00 | 2.9 | 11.7 | 18.8•18.8•18.8•6.3•0.006 | 0.71 | 3.3 | 25.0 |
| GCTRZZnDx | 51.8•51.8•5.2•17.3•0.017 | 1.41 | 2.0 | 8.2 | 26.2•26.2•2.6•8.7•0.009 | 0.85 | 3.5 | 16.7 |

B. Efflux pump

| Drugs | SG | | | | OECM-1 | | | |
|---|---|---|---|---|---|---|---|---|
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| Dx | 0.091 | | | | 0.258 | | | |
| CClZnDx | 24.6•24.6•8.2•0.008 | 0.9 | 17.0 | 11.4 | 18.3•18.3•6.1•0.006 | 0.4 | 28.6 | 43.0 |
| GClZnDx | 33.1•33.1•11.0•0.011 | 0.6 | 12.6 | 8.3 | 33.0•33.0•11.0•0.011 | 0.8 | 15.8 | 23.5 |
| GCClZnDx | 24.0•24.0•24.0•8.0•0.008 | 0.6 | 17.4 | 11.4 | 20.4•20.4•20.4•6.8•0.007 | 0.5 | 25.6 | 36.9 |
| CMemZnDx | >25•25•8.3•0.008 | 1.3 | 2.4 | 11.4 | >75•75•25•0.025 | 2.1 | 1.4 | 10.3 |
| CTRZZnDx | >25•2.5•8.3•0.008 | 1.2 | 2.8 | 11.4 | 30.5•3.1•10.2•0.010 | 1.0 | 2.4 | 25.8 |
| GCMemZnDx | >20•20•20•6.7•0.006 | 0.7 | 3.1 | 15.2 | >60•60•60•20•0.02 | 1.7 | 1.8 | 12.9 |
| GCTRZZnDx | >20•20•2.0•6.7•0.006 | 0.6 | 3.5 | 15.2 | >60•60•6.0•20•0.02 | 2.0 | 1.3 | 12.9 |
| Drugs | A549 | | | | DLD-1 | | | |
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| Dx | >0.3 | | | | >0.3 | | | |
| CClZnDx | 29.4•29.4•9.8•0.010 | 0.2 | 34.0 | 30.0 | 21.0•21.0•7.0•0.007 | 0.2 | 18.3 | 42.9 |
| GClZnDx | 34.9•34.9•11.6•0.012 | 0.6 | 28.7 | 25.0 | 29.6•29.6•9.9•0.10 | 0.9 | 13.0 | 3.0 |
| GCClZnDx | 20.7•20.7•20.7•6.9•0.007 | 0.3 | 48.3 | 42.9 | 19.7•19.7•19.7•6.6•0.007 | 0.4 | 19.5 | 42.9 |
| CMemZnDx | >75•75•25•0.025 | 1.3 | 1.1 | 12.0 | 26.3•26.3•8.8•0.009 | 0.7 | 2.1 | 33.3 |
| CTRZZnDx | >75•7.5•25•0.025 | 1.1 | 1.4 | 12.0 | 48.2•4.8•16.1•0.016 | 1.0 | 1.6 | 18.8 |
| GCMemZnDx | >60•60•60•20•0.02 | 1.6 | 1.4 | 15.0 | 26.8•26.8•26.8•8.9•0.009 | 0.9 | 2.1 | 33.3 |
| GCTRZZnDx | >60•60•6.0•20•0.02 | 1.4 | 1.7 | 15.0 | 38.2•38.2•3.8•12.7•0.013 | 1.1 | 2.0 | 23.1 |

C, curcumin; G, green tea polyphenol; Cl, celecoxib; Mem,memantine; TRZ,thioridazine; Zn, $ZnSO_4$. (A) EI1 and EI2 are efficacy index of efflux pump inhibition of Cl (Mem or TRZ) and Dx respectively. (B) EI1 and EI2 are efficacy index of anticancer effects of Cl (Mem or TRZ) and Dx respectively.

pump ($Na^+$-$K^+$- ATPase) played an important role in the occurrence of MDR.[22–25] In this study, all of the combinatorial PTM regimens exerted not only anticancer effects but also inhibitions of efflux pump, suggesting the possibility that these regimens were able to overcome the MDR problems. On the other hand, most of cancer patients under chemoradiotherapy were in a state of immunodeficiency and dysbiosis accompanied with infectious diseases. The antibacterial effects of these combinatorial PTM regimens revealed their potentiality to overcome the infectious

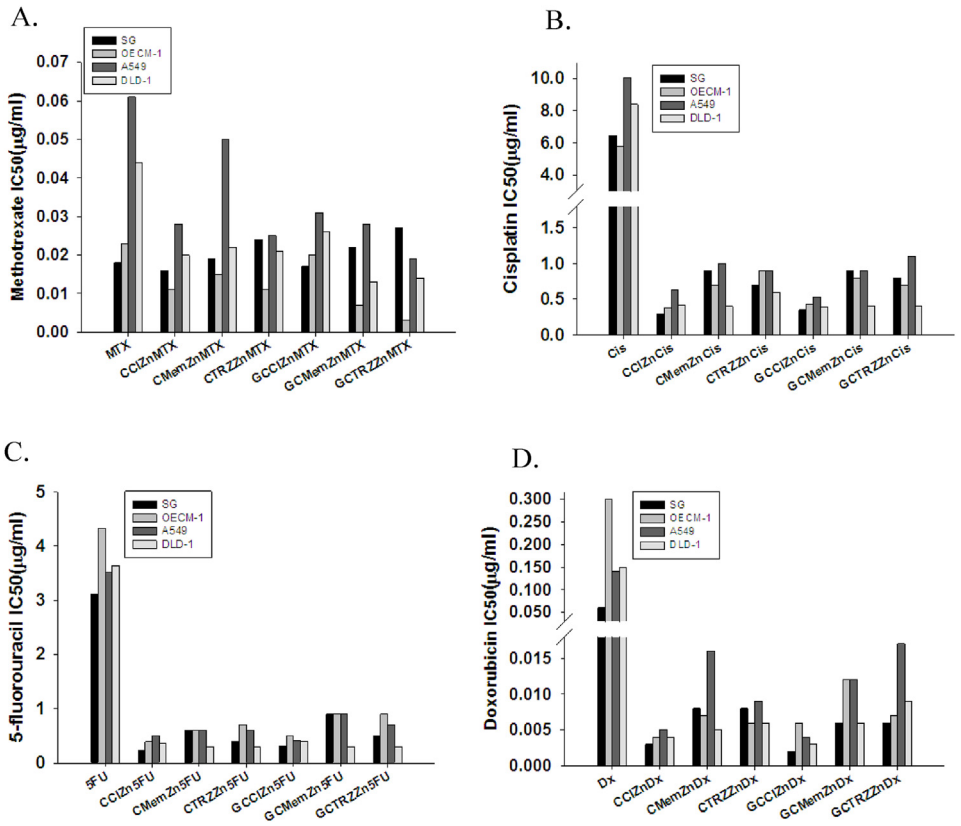


**Figure 2** Increased potency ($IC_{50}$) and selectivity of anticancer effects of methotrexate (A), cisplatin(B), 5-fluorouracil(C) and doxorubicin(D) by various PTM regimens on the cultured cells.

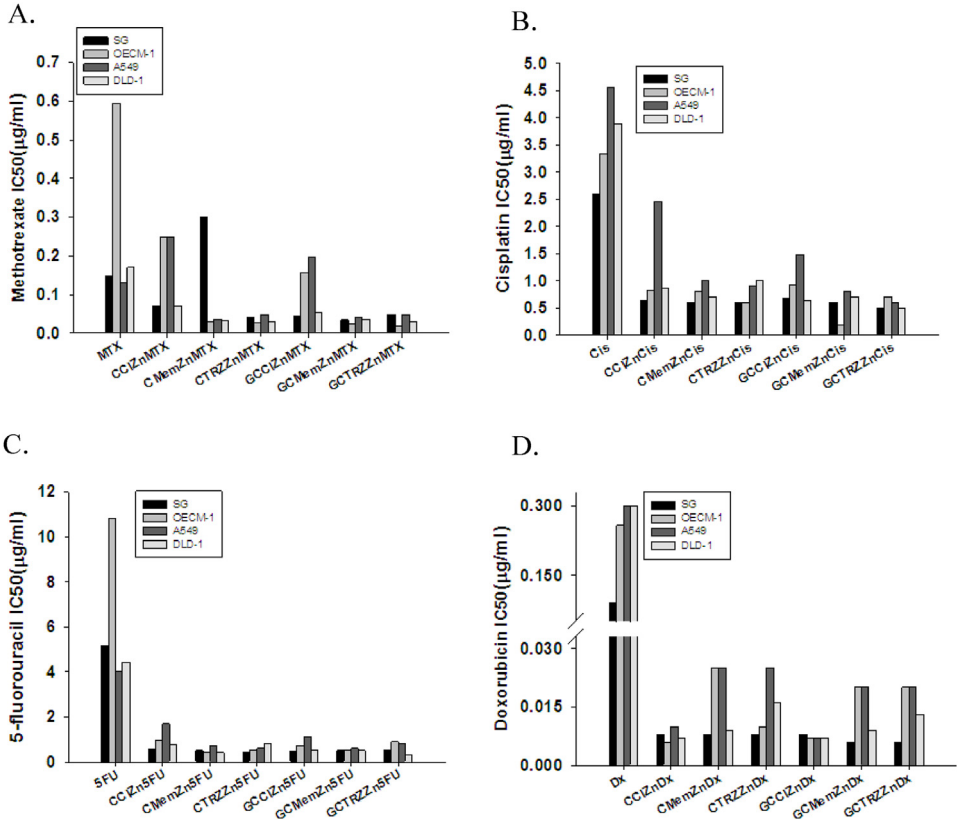


**Figure 3** Increased potency ($IC_{50}$) and selectivity of efflux pump ($Na^+$-$K^+$-ATPase) inhibitions of methotrexate(A), cisplatin(B), 5-fluorouracil(C) and doxorubicin(D) by various PTM regimens on cultured cells

**Table 7** Synergistic antibacterial effects of PTM regimens containing inhibition of biofilm by novel regimens of chemotherapeutic agents on four cultured pathogens.

| Drugs | P.g. | | | | UA159 | | | |
|---|---|---|---|---|---|---|---|---|
| | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 | $IC_{50}$ (μg/ml) | CI | EI1 | EI2 |
| 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 | 0.1 | 8.2 | 5600.0 |
| GCMemZnMTX | 43.8 ± 1.2•43.8 ± 1.2•43.8 ± 1.2•14.6 ± 0.4•14.6 ± 0.4 | 1.2 | 4.0 | 2.1 | 4.4•4.4•4.4•1.5•0.001 | 0.06 | 108.8 | 5600.0 |
| GCTRZZnMTX | 50.3 ± 4.3•50.3 ± 4.3•5.1 ± 0.5•16.8 ± 1.5•16.8 ± 1.5 | 1.4 | 3.9 | 1.8 | 3.6•3.6•0.4•1.2•0.001 | 0.05 | 61.3 | 5600.0 |
| GCClZnCis | >60•60•60•20•2 | >0.8 | <9.8 | <22.5 | 23.8•23.8•23.8•7.9•0.8 | 1.6 | 0.7 | 125.0 |
| GCMemZnCis | 66.5 ± 3.5•66.5 ± 3.5•66.5 ± 3.5•22 ± 1.0•2 ± 0 | 1.2 | 2.6 | 22.5 | 68 ± 7•68 ± 7•68 ± 7•22.5 ± 2.5•2 ± 0 | 0.9 | 7.0 | 50.0 |
| GCTRZZnCis | 37.3•37.3•3.7•12.4•1.2 | 0.6 | 5.4 | 37.4 | 8.4•8.4•0.8•2.8•0.3 | 0.1 | 30.6 | 333.3 |
| GCClZn5FU | >60•60•60•20•2 | >0.8 | <9.8 | <1420.4 | 21.6•21.6•21.6•7.2•0.7 | 1.6 | 0.8 | 13.9 |
| GCMemZn5FU | 45.5 ± 3.8•45.5 ± 3.8•45.5 ± 3.8•15.3 ± 1.2•1.6 ± 0.3 | 0.8 | 3.9 | 1775.5 | 26.1 ± 1.9•26.1 ± 1.9•26.1 ± 1.9•8.6 ± 0.5•0.9 ± 0.1 | 0.4 | 18.3 | 10.8 |
| GCTRZZn5FU | 37.7•37.7•3.8•12.6•1.3 | 0.6 | 5.3 | 2185.2 | 21.4•21.4•2.1•7.1•0.7 | 0.4 | 11.7 | 13.9 |
| GCClZnDx | >60•60•60•20•2 | >0.8 | <9.8 | <50.0 | 14.4 ± 1.2•14.4 ± 1.2•14.4 ± 1.2•4.8 ± 0.4•0.5 ± 0 | 1.3 | 1.2 | 3.7 |
| GCMemZnDx | 58.4 ± 6.3•58.4 ± 6.3•58.4 ± 6.3•19.5 ± 2.1•2 ± 0.2 | 1.0 | 3.0 | 50.0 | 27.6 ± 3.5•27.6 ± 3.5•27.6 ± 3.5•9.2 ± 1.2•0.9 ± 0.1 | 0.8 | 17.3 | 2.1 |
| GCTRZZnDx | 67.4•67.4•6.7•22.5•2.2 | 1.1 | 3.0 | 45.5 | 25.6 ± 1•25.6 ± 1•2.6 ± 0.2•8.6 ± 0.3•0.9 ± 0 | 0.9 | 9.4 | 2.1 |
| 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 | <11.3 | <5.0 |
| GCMemZnMTX | 13.9•13.9•13.9•4.6•4.6 | 0.2 | 20.2 | 21.7 | 24.5•24.5•24.5•8.2•8.2 | 0.3 | 26.1 | 12.2 |
| GCTRZZnMTX | 19.3•19.3•1.9•6.4•6.4 | 0.2 | 157.9 | 15.6 | 31.8•31.8•3.2•10.6•10.6 | 0.4 | 11.1 | 9.4 |
| GCClZnCis | 19.9•19.9•19.9•6.6•0.7 | 0.2 | 50.3 | 24.9 | >60•60•60•20•2 | >0.6 | <11.3 | <50.0 |
| GCMemZnCis | 30 ± 6•30 ± 6•30 ± 6•10 ± 2•1 ± 0 | 0.4 | 9.4 | 17.4 | 42.5 ± 0.5•42.5 ± 0.5•42.5 ± 0.5•14 ± 0•1 ± 0 | 0.4 | 15.1 | 100.0 |
| GCTRZZnCis | 48.6•48.6•4.9•16.2•1.6 | 0.5 | 61.2 | 10.9 | >60•60•6•20•2 | >0.7 | <5.9 | <50.0 |
| GCClZn5FU | 20.3•20.3•20.3•6.8•0.7 | 0.2 | 49.3 | 70.0 | 35.5•35.5•35.5•11.8•1.2 | 0.3 | 19.0 | 185.8 |
| GCMemZn5FU | 32.7 ± 4.2•32.7 ± 4.2•32.7 ± 4.2•10.9 ± 1.6•1 ± 0 | 0.4 | 8.6 | 49.0 | 44.5 ± 4.5•44.5 ± 4.5•44.5 ± 4.5•14.7 ± 1.4•1.7 ± 0.4 | 0.4 | 14.4 | 147.1 |
| GCTRZZn5FU | 53•53•5.3•17.7•1.8 | 0.4 | 56.6 | 27.2 | 6.7•6.7•0.7•2.2•0.2 | 0.1 | 50.9 | 1115.0 |
| GCClZnDx | 18.1 ± 1.8•18.1 ± 1.8•18.1 ± 1.8•6 ± 0.6•0.6 ± 0.1 | 0.2 | 55.2 | 180.3 | >60•60•60•20•2 | >0.6 | <11.3 | <16.0 |
| GCMemZnDx | 25.8 ± 4.1•25.8 ± 4.1•25.8 ± 4.1•8.6 ± 1.4•0.9 ± 0.2 | 0.3 | 10.9 | 120.2 | >60•60•60•20•2 | >0.6 | <10.7 | <16.0 |
| GCTRZZnDx | 23.5 ± 0.9•23.5 ± 0.9•2.4 ± 0.1•7.8 ± 0.3•0.8 ± 0 | 0.2 | 125.0 | 135.3 | >60•60•6•20•2 | >0.7 | <5.9 | <16.0 |

C, curcumin; G, green tea polyphenol; Cl,celecoxib; Mem,memantine; TRZ,thioridazine; Zn, $ZnSO_4$; MTX, methotraxete; Cis,cisplatin; 5FU, 5-fluorouracil; Dx, doxorubicin.
EI1 and EI2 are efficacy index of anti-biofilm formation of Cl (Mem or TRZ) and MTX (Cis, 5FU or Dx) respectively.

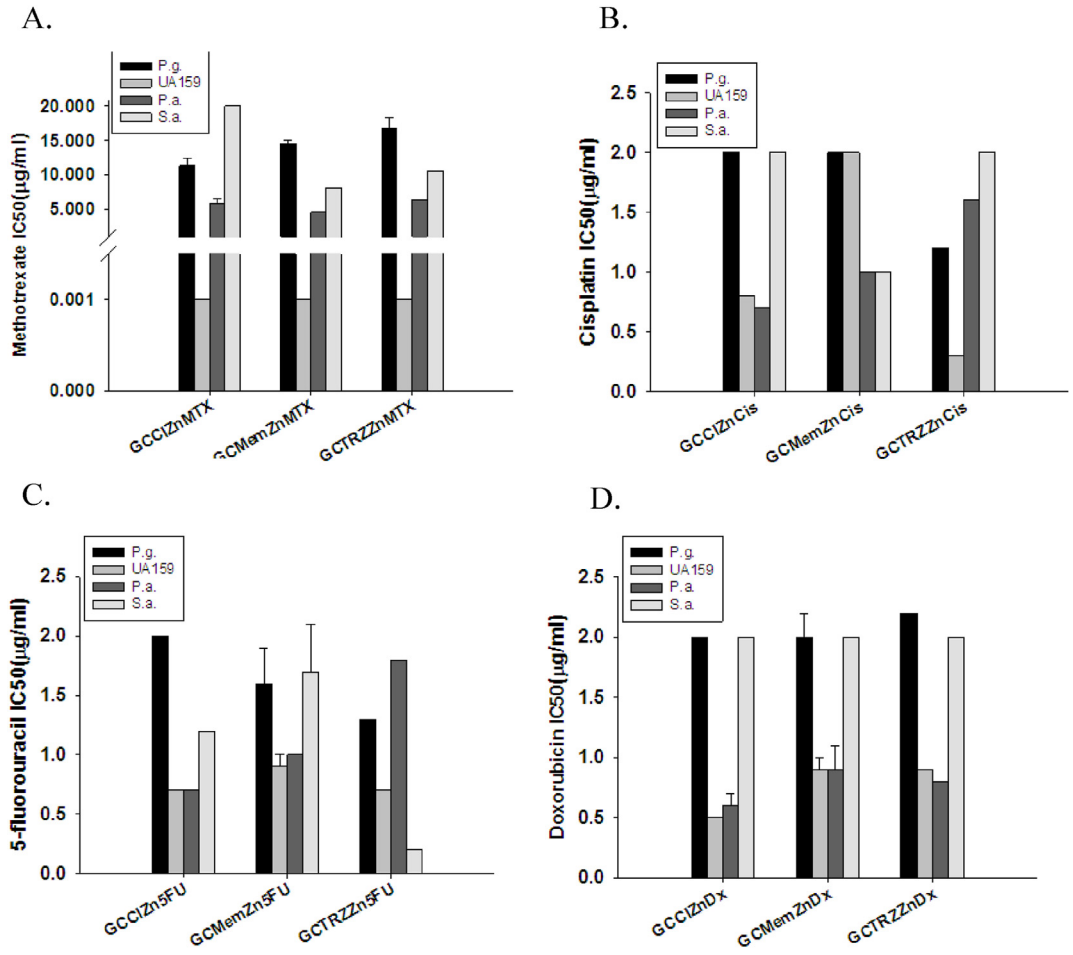


**Figure 4** Synergistic inhibition of biofilm formation by combinatorial regimens of four chemotherapeutic agents (methotrexate(A), cisplatin(B), 5-fluorouracil(C) and doxorubicin(D)) on four cultured pathogens.

problem. However, further clinical studies are needed to make all of the beneficial effects of PTM regimens to become true.

## Declaration of competing interest

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

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