Mixed Alkyltrimethylammonium-Alkylcarboxylate Surfactants Systems: A Potential Anti-Proliferation Agent for MDA-MB-231 Cancer Cell Line

Huei Lim (✉ limwen@mpob.gov.my)

Malaysian Palm Oil Board

Shuang Yong

Universiti Putra Malaysia

Yee Phang

Universiti Putra Malaysia

Hock Chuah

University of Malaya

Noorjahan Alitheen

Universiti Putra Malaysia

Research Article

Keywords: alkyltrimethylammonium-alkylcarboxylate surfactants systems, catanionic, cytotoxicity, withcatanionic, tocotrienol rich fraction

Posted Date: December 8th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-116419/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Mixed alkyltrimethylammonium-alkylcarboxylate Surfactants Systems: A Potential Anti-Proliferation Agent for MDA-MB-231 Cancer Cell Line

Xiou Shuang Yong^{1,2}, Wen Huei Lim^{2*}, Lai Yee Phang¹, Noorjahan Banu Alitheen¹
& Cheng Hock Chuah³

¹Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

²Advanced Oleochemical Technology Division (AOTD), Malaysia Palm Oil Board (MPOB), 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.

³Unit of Research on Lipids (URL), Chemistry Department, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

*Correspondence email: limwen@mpob.gov.my

Abstract

Series of catanionic based alkyltrimethylammonium-alkylcarboxylate surfactants systems having different degree of alkyl chain length asymmetry were prepared and tested for their cytotoxicity against both fibroblast 3T3 and breast cancer MDA-MB-231 cell lines. Catanionic surfactant with the highest alkyl chain length possessed significant potent cytotoxicity, with a half maximal inhibitory concentration (IC_{50}) of $4.04 \pm 0.06 \mu M$ on MDA-MB-231 cell line. Combination of anti-cancer agent (tocotrienol rich fraction, TRF; and curcumin, respectively) with the catanionic at different weight ratios was found to exert lower cytotoxicity on both cell lines as compared to the treated cells with catanionic only. Noticeably, combination of TRF with catanionic showed higher anti-cancer activities than the incorporation of curcumin with catanionic (resulted low IC_{50} value). Combination index (CI) of both anti-cancer agent (TRF and curcumin) and catanionic mixtures indicated antagonistic effect (more than 1), respectively, which may explained catanionic surfactant having long alkyl chain length has the potential to act as anti-cancer agent in its pristine form and less effective with other anti-cancer agents combination.

Introduction

As it is widely known, surface active agents or surfactants having unique properties are been enormously utilized in cosmetic and personal care, chemical processes as well as health and pharmaceutical sectors ¹. Apart from the common classes of surfactants (anionic, cationic, nonionic and zwitterionic), there is a new class of surfactant known as catanionic surfactants that are produced from mixing of two oppositely charged ionic surfactants at equimolar ratio ². This type of surfactant exhibited very interesting properties and unique self-aggregation behaviour into

various aggregates such as vesicles, micelles, hollow icosahedra and planar bilayers³⁻⁵.

Interestingly, a recent review highlighted that the vesicles formed in cationic surfactants have been correlated with anti-cancer activities *in vitro*⁶, representing a useful finding for pharmaceutical industries. Treatment with cationic surfactant vesicles produced from sodium dodecylsulfate and hexadecyltrimethylammonium bromide (TA₁₆) induced apoptosis in murine macrophage-like cells but no apoptosis occurred in normal cells⁷. The cell proliferation of cancer cells was much more inhibited than that of normal mouse fibroblast cells when treated with cationic surfactant vesicles, this was explained by different intrinsic cell membrane permeability of cancer cells in relative to normal cells⁸. Besides that, synthesis of chlorambucil prodrug-containing cationic vesicles that acted as both carrier and active ingredients at the same time exhibited desirable prolonged drug release and enhanced anti-cancer activities³. On the other hand, cationic solid lipid nanoparticles constructed from cationic microemulsion efficiently transported chemotherapy drug across blood brain barrier for the treatment of malignant brain tumour in central nervous system^{9,10}. Novel cationic microemulsion containing curcumin displayed higher cytotoxicity *in vitro* and effectively suppressed tumour growth *in vivo* through intravenous or oral administrations¹¹. Further studies are required to support the anti-cancer activities of cationic surfactants.

Cancer is a disease with immense public health significance suffered by over millions of patients in the world. Breast cancer is the most frequent invasive

cancer among women in both developed and developing countries ¹². Breast cancer is a highly metastatic cancer which is capable to spread from one site to another site very rapidly. Triple negative breast cancer accounts for 15% to 20% of all breast cancers ¹³. The treatment for this highly invasive triple negative breast cancer is very challenging as there is still lacking of targeted treatment site for targeted therapy and available targeted therapeutic agent ¹⁴. Furthermore, adverse effects of cancer treatment and anti-cancer drugs that damages both tumour and normal cells at the same time still remained unresolved ¹⁵. A recent review concluded that 70% of articles reported enhanced chemotherapy efficiency with the presence of antioxidants ¹⁵. This makes antioxidants such as tocotrienol, quercetin, curcumin, carotene, vitamin C, glutathione, coenzyme Q10 and selenium gaining popularity in anti-cancer researches ¹⁵⁻¹⁸.

Other than that, hexadecanoic acid (C₁₆) and octadecanoic acid (C₁₈) which are found abundantly in palm oil fractions were discovered to have anti-cancer activities ¹⁹⁻²¹. C₁₆ and C₁₈ were found to inhibit the colony-forming ability of certain cancer cells *in vitro* and inhibit tumour development *in vivo* ²²⁻²⁴, but not the healthy normal fibroblasts in dose-dependent manner ²². Studies showed the inhibition of cancer cell proliferation of C₁₆ and C₁₈ was caused by apoptosis ^{19,20}. However, another study demonstrated C₁₈ inhibited cancer cell proliferation by inhibiting cell adhesion that involved a laminin integrin receptor mechanism ²⁵. Since carboxylic acids were insoluble in aqueous, limited studies focused on anti-cancer activities of carboxylic acids. Carboxylic acids have been employed as biodegradable surfactants reacted with drugs to form catanionic vesicles for drug delivery systems ²⁶.

So far, involvement of palm-based carboxylic acids and quaternary ammonium surfactants in cationic surfactants is scarcely reported and has not been investigated extensively. Additionally, limited studies reported *in vitro* cytotoxicity of cationic surfactant. Cytotoxicity evaluation of starting materials including surfactants was essential as a beginning step in cancer research studies. In the present study, cytotoxicity of cationic surfactants systems produced from mixtures of carboxylic acids and alkyltrimethylammonium bromide (TA_m) or alkyltrimethylammonium chloride (TAC_m) on both mouse embryo fibroblast and human breast cancer cell lines were investigated. Combination of selected cationic surfactant with tocotrienol rich fraction (TRF) and curcumin as anti-cancer agents were examined on both cell lines, respectively.

Methods

Chemicals and Reagents

Octanoic acid (C_8), decanoic acid (C_{10}), dodecanoic acid (C_{12}), tetradecanoic acid (C_{14}), C_{16} and C_{18} were provided by Wilmar Pasir Gudang Edible Oils Sdn. Bhd. (Johor, Malaysia). TRF containing 22.8% α -tocopherol, 25.1% α -tocotrienol, 2.7% β -tocotrienol, 36.8% γ -tocotrienol and 12.5% δ -tocotrienol was obtained from Supervitamins Sdn. Bhd., (Johor, Malaysia). Curcumin ($\geq 94\%$ curcuminoid and $\geq 80\%$ curcumin), TA_m and TAC_m where $m = 12, 14, 16$ and 18 , sodium bicarbonate, dimethyl sulfoxide, fetal bovine serum and high glucose Dulbecco's modified Eagle medium containing 4500 mg L^{-1} glucose and 584 mg L^{-1} L-glutamine were purchased from Sigma-Aldrich (Missouri, USA). Recombinant trypsin (TrypLETM Express Enzyme) and penicillin-streptomycin solution containing $10,000 \text{ units mL}^{-1}$ of penicillin and $10,000 \text{ } \mu\text{g mL}^{-1}$ of streptomycin were purchased

from Gibco (New York, USA). Phosphate buffered saline tablets was purchased from Oxoid (Hampshire, UK) while 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Invitrogen (New York, USA). All chemicals used in this study were of analytical grade unless stated otherwise.

Preparation of Catanionic Surfactants

Neat catanionic systems were prepared by using a modified version of a previously published procedure²⁷. In brief, an equimolar ratio of cationic surfactant (TA_m and TAC_m , where $m = 12, 14, 16$ and 18) and anionic surfactant (C_n , where $n = 8, 10, 12, 14, 16$ and 18) were weighed and dissolved together in chloroform. The mixture was allowed to undergo complexation and then isolated as a solid white precipitate by fully evaporated off the chloroform. In the preparation of catanionic stock solution, neat catanionic surfactant was weighed and added into volumetric flask with distilled water, respectively. The catanionic stock solution was kept in water bath with magnetic stirrer at temperature below 55°C to ensure neat catanionic surfactant was fully dissolved. The surfactants were sterilized by filtration using $0.2\ \mu\text{M}$ pore size cellulose acetate syringe filters prior to use.

Cell Lines and Cell Culture

The mouse embryo fibroblast cell line 3T3 was obtained from cell bank of International Medical University (Kuala Lumpur, Malaysia) while human breast cancer cell line MDA-MB-231 (ATCC. HTB-26) was purchased from the American Type Culture Collection (Virginia, USA). Both cell lines were maintained in high

glucose Dulbecco's modified Eagle medium, supplemented with 3700 mg mL⁻¹ sodium bicarbonate, 10% of heat-inactivated fetal bovine serum and 1% of penicillin-streptomycin solution. Cells were cultured in 25 cm² cell flask in a humidified atmosphere containing 5% of carbon dioxide at 37°C.

Cell Seeding and Treatment

A 100 µL of medium containing 1×10^4 cells was seeded into each well of 96-well microplate and incubated for 24 hours prior to treatment. On the following day, both cell lines were treated with seven different concentrations of individual parent surfactants, cationic surfactants, anti-cancer agents (TRF and curcumin) and mixtures of selected cationic surfactant with anti-cancer agent. Cationic surfactants were synthesized and prepared as mentioned above. 2 mM of TA_m, TAC_m and cationic surfactants were prepared from stock solutions and then further diluted to achieve final concentrations 0 µM to 100 µM with fresh media. Due to limited solubility of anionic surfactants, TRF and curcumin, they were freshly dissolved in ethanol prior to use. Stock solutions were prepared at concentration of 100 mM for C_n, 25 mg mL⁻¹ for TRF and curcumin. C_n where $n = 8, 10, 12, 14, 16$ and 18 were further diluted with fresh media into final concentration of 0 µM to 500 µM while TRF and curcumin were at final concentration of 125 µg mL⁻¹, respectively. The final concentration of ethanol in the culture medium was always never exceeded 0.5%. Highest concentration of 0.5% ethanol in working solutions have been proven not toxic to the cells previously²⁸. Cationic surfactant with the highest potential anti-cancer activities among the cationic surfactants systems was selected for combination with TRF and curcumin, respectively. Based on MTT assay, C₁₈TA₁₈ was selected and different weight ratios of TRF:C₁₈TA₁₈ (1:1, 1:3, 1:5, 1:10,

1:20 and 1:30) and curcumin:C₁₈TA₁₈ (1:5, 1:10, 1:20 and 1:30) were prepared in according to their solubility limitation. The desired amount of TRF:C₁₈TA₁₈ and curcumin:C₁₈TA₁₈ mixtures at respective ratios were weighted and dissolved in aqueous solution at 2 mM, followed by sonication at room temperature for 1 hour. The stock solutions were further diluted with fresh media to achieve final concentrations of 0 μ M to 100 μ M. After subjected to different treatments as described above, the treated cells were incubated for 24 hours post-treatment. Each assay and concentration were tested in triplicate (n = 3). Control cells were exposed to culture medium only. The cell density after 24 hours post-treatment was observed using Zeiss Primovert inverted light microscope (Jena, Germany).

MTT Cytotoxicity Assay

The cytotoxicity of individual parent surfactants, cationic surfactants, TRF, curcumin, different weight ratios of C₁₈TA₁₈:anti-cancer agent (namely, TRF and curcumin) towards MDA-MB-231 and 3T3 cell lines were determined via MTT assay as described by Mosmann²⁹. The cell viability of treatment cells was compared relatively to untreated control cells. After 24 hours of post-treatment, a 20 μ L of 5 mg mL⁻¹ MTT reagent was added into each well and then the cells were further incubated for 3 hours in humidified atmosphere with 5% of carbon dioxide at 37°C. Subsequently, 100 μ L of dimethyl sulfoxide was added into each well to completely dissolve the formazan crystals. The absorbance was read at 570 nm using Synergy H1 Hybrid Multi-Mode Reader (Vermont, USA). The results of cytotoxicity assay were expressed as half maximal inhibitory concentration (IC₅₀), calculated by fitting experimental data into a dose-response curve. The results were calculated as the percentage of cell viability in relative to untreated cells as control with 100%

viability. The percentage of cell viability was calculated according to the following formula:

$$\text{Percentage of cell viability} = \frac{\text{Absorbance of treated cells at 570 nm}}{\text{Absorbance of untreated cells at 570 nm}} \times 100 \text{ Equation (1)}$$

Combination Index Analysis

Combination Index of TRF:C₁₈TA₁₈ and curcumin:C₁₈TA₁₈ was calculated using the method described in previous studies^{30,31}. A combination index of more than, equal or less than 1 were defined as antagonistic, additive and synergistic effect, respectively^{30,32}. The combination index was calculated according to the following formula:

$$\text{Combination Index} = \frac{C_{A,50}}{IC_{50,A}} + \frac{C_{B,50}}{IC_{50,B}} \text{ Equation (2)}$$

Where, C_{A,50} and C_{B,50} are the concentration required for C₁₈TA₁₈ and anti-cancer agent (TRF or curcumin) combination to achieve IC₅₀. IC_{50,A} and IC_{50,B} are the IC₅₀ for single compound to achieve the same effect.

Statistical Analysis

All experiments were performed in triplicates and the results were expressed as means ± standard deviation. The one-way Analysis of Variance was used to determine the statistical significance difference. Tukey's and Bonferroni's Multiple Comparison Test was conducted subsequently for significant main effects. The GraphPad Prism Version 7.04 software was used for statistical analyses. All results were considered to be statistically significant at $p < 0.05$.

Data Availability

All data generated and analysed during this study are included in this article.

Results and Discussion

Cytotoxicity of Parent Surfactants

Tables 1 and 2 show the *in vitro* response of 3T3 and MDA-MB-231 cell lines after treated with different alkyl chain length and counterions parent surfactants (TA_m , TAC_m and C_n) at various concentrations for 24 hours. 3T3 cell line was used as a reference for normal cell line when comparing with cancerous MDA-MB-231 cell line. Overall, cytotoxicity response of both cell lines toward all individual parent surfactants happened in a dose-dependent manner. Regardless of the counterions of parent surfactants, cytotoxicity on both cell line increased with the alkyl chain length of cationic and anionic parent surfactants. Increased cytotoxicity with the alkyl chain length of surfactant have been generally related to higher interactions between surfactant and plasma membrane of cells due to higher hydrophobicity in surfactants, that eventually damaged the cells³³⁻³⁵.

The percentage of cell viability on both cell lines for quaternary ammonium cationic parent surfactants declined drastically from 0 μ M to 100 μ M. However, both cell lines remained more than 50% of cell viability at the concentration of 100 μ M for C_n anionic parent surfactants where $n = 8, 10, 12, 14, 16$ and 18 . This result indicates higher cytotoxicity of quaternary ammonium cationic surfactants compared with carboxylic anionic surfactants. Similar outcomes have been reported where cytotoxicity of anionic surfactants was lower than cationic surfactants^{36,37}. Moreover, it also had been reported that cationic surfactants was capable to induce apoptosis at a concentration lower than critical micelle concentration via mitochondrial

dysfunction but anionic or zwitterionic surfactants were less likely to trigger apoptosis³⁸.

Within two different types of quaternary ammonium cationic parent surfactants, TA_m with bromide counterion displayed a higher cytotoxicity to both cell lines than TAC_m with chloride counterion. Higher cytotoxicity of TA_m resulted a steeper declination in the percentage of cell viability versus concentration curve and lower IC_{50} than TAC_m . IC_{50} of TA_{18} on both cell lines were significantly lower compared with TA_{12} and TA_{14} but not TA_{16} . Besides that, TAC_{18} also gave significantly lower IC_{50} in comparison with other alkyl chain length on 3T3 and MDA-MB-231 cell lines, except TAC_{16} on MDA-MB-231 cell line. Yet, IC_{50} of both TA_m and TAC_m on 3T3 cell lines still relatively higher than that of MDA-MB-231 cell line. The result was in line with previous study in which cationic surfactants having bromide as a counterion was known to have higher cytotoxicity than the one with chloride counterion³⁴.

There was no obvious decline of cell viability when treated with lower alkyl chain length anionic parent surfactants, C_n . Among anionic parent surfactants, only C_{16} and C_{18} achieved IC_{50} within the treatment concentration. In agreement with a previous study that demonstrated saturated carboxylic acids with less than fourteen carbon chain length did not inhibit cell proliferation or induce cytotoxicity³⁵. C_{18} with IC_{50} of $243.16 \mu M \pm 1.48 \mu M$ showed significantly higher cytotoxicity than C_{16} that gave IC_{50} of $419.59 \mu M \pm 3.01 \mu M$ on 3T3 cell line. IC_{50} of C_{16} was around two-fold of that in C_{18} treatment on both cell line. Interestingly, both C_{16} and C_{18} showed approximately two times lower cytotoxicity on 3T3 cell line compared with MDA-

MB-231 cell line. Thus, long chain saturated carboxylic acids particularly C₁₆ and C₁₈ effectively inhibited the growth of MDA-MB-231 cell line, provided anti-cancer activities. This was in agreement with previous studies stating C₁₆ and C₁₈ posed anti-cancer activities and induced apoptosis in cancer cells¹⁹⁻²¹. Carboxylic acids were reported to induce apoptosis via disruption of cell membrane integrity, induced DNA fragmentation as well as decreased mitochondrial membrane potential^{35,39}.

After 24 hours treatment, the cell density of 3T3 and MDA-MB-231 cell line were observed where at the same parent surfactants treatment concentration, 3T3 cell line showed higher cell density than MDA-MB-231 cell line. The cell density after treated with TA_m was lower than that of TAC_m at the same concentration. Even at 125 µM of C_n, the cell density observed was still higher than both cell lines treated with 6.25 µM of cationic parent surfactants. Since cytotoxicity increased with the alkyl chain length of parent surfactants, the cell density appeared to reduce as the alkyl chain length of parent surfactants increased.

Cytotoxicity of Catanionic Surfactants

In many instants, improved physicochemical properties are often observed in catanionic surfactants over the single pure form surfactant. Both cell lines achieved IC₅₀ within treatment concentrations, IC₅₀ of each catanionic surfactant on 3T3 and MDA-MB-231 cell line are observed as indicated in Tables 3 and 4. The findings showed cytotoxicity of catanionic surfactants on both cell lines also happened in a dose-dependent manner, this was in line with cytotoxicity pattern of catanionic vesicles on macrophage-like cells⁷ and drug-participating catanionic aggregates on cancer cell lines³.

The cytotoxicity of parent surfactants was clearly reflected in their resulting catanionic surfactants where catanionic surfactant with bromide counterions and higher alkyl chain length displayed higher cytotoxicity on 3T3 and MDA-MB-231 cell lines. In other words, higher alkyl chain length catanionic surfactants with increased hydrophobicity were able to inhibit the cell proliferation more effectively than the lower alkyl chain length combinations. The IC_{50} of catanionic surfactants on both cell lines were more affected by the change of alkyl chain length in cationic parent surfactants (TA_m and TAC_m) compared with anionic parent surfactants, C_n . This could be related with presence of ammonium group in TA_m and TAC_m ⁴⁰. Furthermore, IC_{50} of catanionic surfactants seems to occur mainly above their respective critical aggregation concentration. This showed that the surfactant aggregates played a crucial role in cytotoxicity⁴¹.

Enhanced effect of catanionic surfactants over their anionic parent surfactants can be easily seen from their IC_{50} , where substantially lower concentration of catanionic surfactants was needed to inhibit the cell proliferation by the same amount. In comparison with cationic parent surfactants, only some of the catanionic surfactants showed enhanced cytotoxicity, particularly for catanionic surfactants with higher alkyl chain length. Even though C_nTA_m series exhibited higher cytotoxicity than C_nTAC_m series, both of these series were still had lower cytotoxicity on 3T3 cell line than MDA-MB-231 cell line. The IC_{50} of catanionic surfactants on 3T3 cell line was 6% to 77% higher than MDA-MB-231 cell line for each corresponding C_nTA_m , but only higher than MDA-MB-231 cell line by a magnitude of 0.4% to 39% for each corresponding C_nTAC_m . The overall differences

in IC_{50} between both cell lines for C_nTAC_m series were still smaller than that in C_nTA_m series.

IC_{50} of all four different alkyl chain length catanionic surfactants comprised of the same anionic parent surfactants were found to have difference statistically on 3T3 cell line except between C_nTA_{16} and C_nTA_{18} as well as between $C_{18}TAC_{16}$ and $C_{18}TAC_{18}$. When the catanionic surfactants comprised of the same anionic surfactants were compared on MDA-MB-231 cell line, IC_{50} of C_nTA_{12} , C_nTA_{14} , C_nTAC_{12} and C_nTAC_{14} were significantly higher across the groups, respectively. In contrary, no statistical differences were observed in IC_{50} of C_nTA_{16} and C_nTA_{18} as well as C_nTAC_{16} and C_nTAC_{18} on MDA-MB-231 cell line across the group for each corresponding anionic parent surfactants series except for $C_{18}TA_{18}$. $C_{18}TA_m$ series was the only series that exhibited IC_{50} which significantly different across all different alkyl chain length cationic parent surfactants on MDA-MB-231 cell line. $C_{18}TA_{18}$ showed significantly lower IC_{50} of $4.04 \mu M \pm 0.06 \mu M$ on MDA-MB-231 cell line across the same anionic surfactant homologous group with IC_{50} of $21.06 \mu M \pm 0.30 \mu M$, $5.17 \mu M \pm 0.17 \mu M$ and $4.61 \mu M \pm 0.11 \mu M$ for $C_{18}TA_{12}$, $C_{18}TA_{14}$ and $C_{18}TA_{16}$, respectively.

From the above, $C_{18}TA_{16}$ and $C_{18}TA_{18}$ were discovered to have the strongest anti-cancer activities as the lowest concentration was required to inhibit the cell proliferation of MDA-MB-231 cell line. In Figure 1, C_8TA_{16} , $C_{10}TA_{16}$, C_8TA_{18} and $C_{10}TA_{18}$ promote the cell proliferation of MDA-MB-231 cell line significantly compared with their cationic parent surfactants, rather than cell inhibition. IC_{50} of $C_{18}TA_{16}$ and $C_{18}TA_{18}$ on MDA-MB-231 cell line were significantly lower than their

corresponding cationic parent surfactants. In comparison with 3T3 cell line, C₁₈TA₁₆ and C₁₈TA₁₈ significantly inhibited cell proliferation on MDA-MB-231 cell line (Figure 2). As mentioned earlier, C₁₈TA₁₈ inhibited MDA-MB-231 cell line to the highest extent. This indicated the potential of C₁₈TA₁₈ to be used as anti-cancer agent in certain medical and pharmaceutical areas since lower amount was required than traditional surfactant is one of the importance.

Combination Analysis of C₁₈TA₁₈ With Other Anti-Cancer Agents

Cytotoxicity of TRF and curcumin as anti-cancer agents are investigated and shown in Table 5. Similar as previous studies, TRF and curcumin effectively inhibited cell proliferation of cancerous cell compared with healthy normal cell¹⁶⁻¹⁸. From the results, TRF displayed significantly higher cytotoxicity than curcumin on both cell lines. IC₅₀ of TRF on 3T3 cell line was 28.39 $\mu\text{g mL}^{-1} \pm 0.89 \mu\text{g mL}^{-1}$, slightly higher than that of MDA-MB-231 cell line with IC₅₀ of 22.77 $\mu\text{g mL}^{-1} \pm 0.34 \mu\text{g mL}^{-1}$. Treatment with TRF on MDA-MB-231 cell line gave IC₅₀ that was almost twice lower than that in curcumin. Results indicated that TRF was more potent in the inhibition of cancer cell proliferation than curcumin.

In the previous section, C₁₈TA₁₈ was revealed as the most potent cationic surfactant on MDA-MB-231 cell line. Thus, C₁₈TA₁₈ was selected to be incorporated with TRF and curcumin for combination cytotoxicity evaluation, respectively. Cytotoxicity of different weight ratios of TRF:C₁₈TA₁₈ and curcumin:C₁₈TA₁₈ on 3T3 and MDA-MB-231 cell lines is illustrated in Figure 3. TRF:C₁₈TA₁₈ and curcumin:C₁₈TA₁₈ at all weight ratios displayed significantly lower cytotoxicity than treatment with C₁₈TA₁₈ alone in both 3T3 and MDA-MB-231 cell lines. 3T3 cell line

did not have remarkable difference in cytotoxicity between different weight ratios of TRF:C₁₈TA₁₈ (IC₅₀ ranging from 25 μ M to 23 μ M), except 1:1 weight ratio (IC₅₀ = 27.01 μ M $\pm$ 0.86 μ M). These IC₅₀ were 3.9-fold to 4.6-fold higher than that in the treatment with C₁₈TA₁₈. Yet, IC₅₀ were significantly different between different weight ratios of curcumin:C₁₈TA₁₈, except between 1:5 and 1:10 weight ratio. In comparison with C₁₈TA₁₈, the cytotoxicity after incorporating curcumin on 3T3 cell line was only 1.8-fold to 3.5-fold lower.

For MDA-MB-231 cell line, 1:30 weight ratio of TRF:C₁₈TA₁₈ and curcumin:C₁₈TA₁₈ showed the highest cytotoxicity which also exhibited significantly higher cytotoxicity across the experimental weight ratios except 1:20 weight ratio. IC₅₀ of 1:30 TRF:C₁₈TA₁₈ weight ratio was 4.88 μ M $\pm$ 0.28 μ M and 9.13 μ M $\pm$ 0.55 μ M for 1:30 curcumin:C₁₈TA₁₈ weight ratio on MDA-MB-231 cell line. When higher amount of respective TRF and curcumin was incorporated into C₁₈TA₁₈, IC₅₀ of MDA-MB-231 cell line inclined significantly to 10.31 μ M $\pm$ 0.20 μ M at 1:1 TRF:C₁₈TA₁₈ weight ratio and 12.35 μ M $\pm$ 0.22 μ M at 1:5 curcumin:C₁₈TA₁₈ weight ratio. Even though the cytotoxicity was lower after incorporation of anti-cancer agents, 1:20 and 1:30 TRF:C₁₈TA₁₈ still showing enhanced cytotoxicity over their parent surfactants on MDA-MB-231 cell line.

As clearly seen in Figure 3b, incorporation of TRF into C₁₈TA₁₈ showed much lower IC₅₀ than incorporation of curcumin. This indicated that incorporation of TRF into C₁₈TA₁₈ resulted more favourable anti-cancer activities than curcumin on MDA-MB-231 cell line. Although cytotoxicity on MDA-MB-231 cell line reduced after incorporation of anti-cancer agents into C₁₈TA₁₈, the IC₅₀ still showed improved

anti-cancer activities compared with IC_{50} of an anti-cancer prodrug-participating cationic aggregates on leukaemia and breast cancer cell lines in recent study ³. Noticeably, IC_{50} of different weight ratios of TRF:C₁₈TA₁₈ were found to be at least 50% lower than that on 3T3 cell line under the same treatment. Hence, different weight ratios of TRF:C₁₈TA₁₈ showed potential anti-cancer activities.

The combination index of different weight ratios of TRF:C₁₈TA₁₈ and curcumin:C₁₈TA₁₈ was calculated based on Chou and Talalay's Combination Index equation and shown in Table 6. Unfortunately, none of the experimental combinations provide synergistic effects on inhibition of cancer cell proliferation. Therefore, respective incorporation of TRF and curcumin as anti-cancer agents with C₁₈TA₁₈ did not further enhance the anti-cancer activities on MDA-MB-231 cell line. However, the presence of very minute quantity of TRF and curcumin that had been well-recognized to possess antioxidant properties greatly reduced the cytotoxicity of C₁₈TA₁₈ on 3T3 cell line especially with the incorporation of TRF.

Effects of different weight ratios of TRF:C₁₈TA₁₈ and curcumin:C₁₈TA₁₈ in respect to untreated control cells are also revealed in the resulted cell density. At 6.25 μ M of different weight ratios of TRF:C₁₈TA₁₈ and curcumin:C₁₈TA₁₈, 3T3 cell line remained with good cell confluency under inverted microscope. Conversely, the cell viability of MDA-MB-231 cell line was relatively low where low cell density was observed at 6.25 μ M of TRF:C₁₈TA₁₈ and curcumin:C₁₈TA₁₈ weight ratio. Besides that, incorporation of TRF into C₁₈TA₁₈ resulted lower cell density than that of curcumin at the same treatment ratio. Therefore, TRF was more suitable to be

incorporated with C₁₈TA₁₈ in order to kill the cancer cells at greatest magnitude but with minimal damage on healthy cells.

Conclusion

The present study suggests catanionic surfactant is able to inhibit the proliferation of cancerous MDA-MB-231 cell line more effectively than all their anionic parent surfactants and some of their cationic parent surfactants. Among all catanionic surfactants, C₁₈TA₁₈ had significantly inhibited the proliferation of MDA-MB-231 cell line to the highest extend. Respective incorporation of TRF and curcumin into C₁₈TA₁₈ did not further improve the *in vitro* cytotoxicity on MDA-MB-231 cell line. Yet, the cytotoxicity of C₁₈TA₁₈ on 3T3 cell line was significantly reduced after incorporation of TRF and curcumin into C₁₈TA₁₈, respectively. This shows the potential of C₁₈TA₁₈ to be used as an anti-cancer agent in its pristine form as well as with other anti-cancer agents.

References

- 1 Tadros, T. *Encyclopedia of Colloid and Interface Science*. (Springer Berlin Heidelberg, 2013).
- 2 Jokela, P., Joensuu, B. & Khan, A. Phase equilibria of catanionic surfactant-water systems. *The Journal of Physical Chemistry* **91**, 3291-3298, doi:10.1021/j100296a037 (1987).
- 3 Jiang, Y., Hu, X., Zhang, J., Jin, G. & Luan, Y. Chlorambucil prodrug-participating catanionic aggregates for sustained drug release and improved antitumour activity. *Journal of Molecular Liquids* **274**, 556-561, doi:<https://doi.org/10.1016/j.molliq.2018.10.165> (2019).
- 4 Dubois, M. *et al.* Self-assembly of regular hollow icosahedra in salt-free catanionic solutions. *Nature* **411**, 672-675, doi:10.1038/35079541 (2001).
- 5 Hao, J. & Hoffmann, H. Self-assembled structures in excess and salt-free catanionic surfactant solutions. *Current Opinion in Colloid and Interface Science* **9**, 279-293, doi:<https://doi.org/10.1016/j.cocis.2004.06.004> (2004).
- 6 Dhawan, V. V. & Nagarsenker, M. S. Catanionic systems in nanotherapeutics – Biophysical aspects and novel trends in drug delivery applications. *Journal of Controlled Release* **266**, 331-345, doi:<https://doi.org/10.1016/j.jconrel.2017.09.040> (2017).
- 7 Kuo, J.-H. S., Jan, M.-S., Chang, C.-H., Chiu, H.-W. & Li, C.-T. Cytotoxicity characterization of catanionic vesicles in RAW 264.7 murine macrophage-like cells. *Colloids and Surfaces B: Biointerfaces* **41**, 189-196, doi:<http://dx.doi.org/10.1016/j.colsurfb.2004.12.008> (2005).
- 8 Aiello, C., Andreozzi, P., La Mesa, C. & Risuleo, G. Biological activity of SDS-CTAB cat-anionic vesicles in cultured cells and assessment of their cytotoxicity ending in apoptosis. *Colloids and Surfaces B: Biointerfaces* **78**, 149-154, doi:<https://doi.org/10.1016/j.colsurfb.2010.02.013> (2010).
- 9 Kuo, Y.-C. & Hong, T.-Y. Delivering etoposide to the brain using catanionic solid lipid nanoparticles with surface 5-HT-moduline. *International Journal of Pharmaceutics* **465**, 132-142, doi:<https://doi.org/10.1016/j.ijpharm.2014.02.008> (2014).
- 10 Kuo, Y.-C. & Wang, C.-C. Carmustine-loaded catanionic solid lipid nanoparticles with serotonergic 1B receptor subtype antagonist for *in vitro* targeted delivery to inhibit brain cancer growth. *Journal of the Taiwan Institute of Chemical Engineers* **46**, 1-14, doi:<https://doi.org/10.1016/j.jtice.2014.08.035> (2015).
- 11 Li, S. *et al.* Catanionic lipid nanosystems improve pharmacokinetics and anti-lung cancer activity of curcumin. *Nanomedicine: Nanotechnology, Biology and Medicine* **12**, 1567-1579, doi:<https://doi.org/10.1016/j.nano.2016.02.007> (2016).
- 12 Rivera-Franco, M. M. & Leon-Rodriguez, E. Delays in Breast Cancer Detection and Treatment in Developing Countries. *Breast Cancer: Basic and Clinical Research* **12**, 1178223417752677, doi:10.1177/1178223417752677 (2018).
- 13 Brouckaert, O., Wildiers, H., Floris, G. & Neven, P. Update on triple-negative breast cancer: prognosis and management strategies. *International Journal of Women's Health* **4**, 511-520, doi:10.2147/IJWH.S18541 (2012).
- 14 Mehanna, J., Haddad, F. G., Eid, R., Lambertini, M. & Kourie, H. R. Triple-negative breast cancer: current perspective on the evolving therapeutic

- landscape. *International Journal of Women's Health* **11**, 431-437, doi:10.2147/IJWH.S178349 (2019).
- 15 Singh, K., Bhor, M., Kasu, Y. A., Bhat, G. & Marar, T. Antioxidants as precision weapons in war against cancer chemotherapy induced toxicity - Exploring the armoury of obscurity. *Saudi Pharmaceutical Journal* **26**, 177-190, doi:10.1016/j.jsps.2017.12.013 (2018).
- 16 Comitato, R., Ambra, R. & Virgili, F. Tocotrienols: A Family of Molecules with Specific Biological Activities. *Antioxidants* **6**, doi:10.3390/antiox6040093 (2017).
- 17 Nesaretnam, K., Meganathan, P., Veerasenan, S. D. & Selvaduray, K. R. Tocotrienols and breast cancer: the evidence to date. *Genes and Nutrition* **7**, 3-9, doi:10.1007/s12263-011-0224-z (2012).
- 18 Wilken, R., Veena, M. S., Wang, M. B. & Srivatsan, E. S. Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. *Molecular cancer* **10**, 12, doi:10.1186/1476-4598-10-12 (2011).
- 19 Hardy, S. *et al.* Saturated Fatty Acid-induced Apoptosis in MDA-MB-231 Breast Cancer Cells: A ROLE FOR CARDIOLIPIN. *Journal of Biological Chemistry* **278**, 31861-31870, doi:10.1074/jbc.M300190200 (2003).
- 20 Evans, L. M., Cowey, S. L., Siegal, G. P. & Hardy, R. W. Stearate preferentially induces apoptosis in human breast cancer cells. *Nutrition and Cancer* **61**, 746-753, doi:10.1080/01635580902825597 (2009).
- 21 Khan, A. A., Alanazi, A. M., Jabeen, M., Chauhan, A. & Abdelhameed, A. S. Design, synthesis and *in vitro* anticancer evaluation of a stearic acid-based ester conjugate. *Anticancer research* **33**, 2517-2524 (2013).
- 22 Habib, N. A. *et al.* Stearic acid and carcinogenesis. *British Journal of Cancer* **56**, 455-458, doi:10.1038/bjc.1987.223 (1987).
- 23 Fermor, B. F. *et al.* Fatty acid composition of normal and malignant cells and cytotoxicity of stearic, oleic and sterculic acids *in vitro*. *European Journal of Cancer* **28**, 1143-1147, doi:[https://doi.org/10.1016/0959-8049\(92\)90475-H](https://doi.org/10.1016/0959-8049(92)90475-H) (1992).
- 24 Wickramasinghe, N. S., Jo, H., McDonald, J. M. & Hardy, R. W. Stearate inhibition of breast cancer cell proliferation. A mechanism involving epidermal growth factor receptor and G-proteins. *The American journal of pathology* **148**, 987-995 (1996).
- 25 Singh, R. K. *et al.* Stearate inhibits human tumor cell invasion. *Invasion & metastasis* **15**, 144-155 (1995).
- 26 Jiang, Y., Luan, Y., Qin, F., Zhao, L. & Li, Z. Catanionic vesicles from an amphiphilic prodrug molecule: a new concept for drug delivery systems. *RSC Advances* **2**, 6905-6912, doi:10.1039/C2RA20653F (2012).
- 27 Gavrilko, T. *et al.* FTIR and DSC Studies of Binary Mixtures of Long-Chain Aliphatic Compounds: Lauric Acid–Cetyl-trimethylammonium Bromide. *Ukrainian Journal of Physics* **63**, 413-424 (2018).
- 28 Siddiqui, R. A. *et al.* Docosahexaenoic acid induces apoptosis in Jurkat cells by a protein phosphatase-mediated process. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1499**, 265-275 (2001).
- 29 Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods* **65**, 55-63 (1983).

- 30 Chou, T.-C. Theoretical Basis, Experimental Design, and Computerized Simulation of Synergism and Antagonism in Drug Combination Studies. *Pharmacological Reviews* **58**, 621-681, doi:10.1124/pr.58.3.10 (2006).
- 31 Aldhirgham, T., Henderson, K., Nigam, P. & Owusu-Apenten, R. K. A Combination of Curcumin from Turmeric and Alpha-linolenic Acid Shows Antagonism with MCF-7 Breast Cancer Cells in Phenol-red Free Medium. *Journal of Applied Life Sciences International* **10**, 1-12 (2016).
- 32 Chou, T.-C. The combination index ($CI < 1$) as the definition of synergism and of synergy claims. *Synergy* **7**, 49-50, doi:<https://doi.org/10.1016/j.synres.2018.04.001> (2018).
- 33 Zhang, S. *et al.* Antibacterial Activity, *in Vitro* Cytotoxicity, and Cell Cycle Arrest of Gemini Quaternary Ammonium Surfactants. *Langmuir* **31**, 12161-12169, doi:10.1021/acs.langmuir.5b01430 (2015).
- 34 Inacio, A. S. *et al.* *In vitro* surfactant structure-toxicity relationships: implications for surfactant use in sexually transmitted infection prophylaxis and contraception. *PLoS One* **6**, e19850, doi:10.1371/journal.pone.0019850 (2011).
- 35 Harvey, K. A. *et al.* Long-chain saturated fatty acids induce pro-inflammatory responses and impact endothelial cell growth. *Clinical Nutrition* **29**, 492-500, doi:10.1016/j.clnu.2009.10.008 (2010).
- 36 Vlachy, N., Touraud, D., Heilmann, J. & Kunz, W. Determining the cytotoxicity of catanionic surfactant mixtures on HeLa cells. *Colloids and Surfaces B: Biointerfaces* **70**, 278-280, doi:<https://doi.org/10.1016/j.colsurfb.2008.12.038> (2009).
- 37 Sanchez, L., Mitjans, M., Infante, M. R. & Vinardell, M. P. Potential irritation of lysine derivative surfactants by hemolysis and HaCaT cell viability. *Toxicology Letters* **161**, 53-60, doi:<https://doi.org/10.1016/j.toxlet.2005.07.015> (2006).
- 38 Enomoto, R. *et al.* Cationic Surfactants Induce Apoptosis in Normal and Cancer Cells. *Annals of the New York Academy of Sciences* **1095**, 1-6, doi:10.1196/annals.1397.001 (2007).
- 39 Martins de Lima, T., Cury-Boaventura, Maria F., Giannocco, G., Nunes, Maria T. & Curi, R. Comparative toxicity of fatty acids on a macrophage cell line (J774). *Clinical Science* **111**, 307-317, doi:10.1042/cs20060064 (2006).
- 40 Colomer, A. *et al.* pH-Sensitive Surfactants from Lysine: Assessment of Their Cytotoxicity and Environmental Behavior. *Langmuir* **28**, 5900-5912, doi:10.1021/la203974f (2012).
- 41 Egorova, E. M. & Kaba, S. I. The effect of surfactant micellization on the cytotoxicity of silver nanoparticles stabilized with aerosol-OT. *Toxicology in Vitro* **57**, 244-254, doi:<https://doi.org/10.1016/j.tiv.2019.03.006> (2019).

Acknowledgements

The authors gratefully acknowledge Malaysian Palm Oil Board for the support of this work.

Author Contributions

X.S.Y. and W.H.L. designed and developed the synthesis of cationic surfactants described in this paper. X.S.Y. conducted and analysed the cytotoxicity experiment with the supervising of L.Y.P. and N.B.M.A. W.H.L. and L.Y.P. discussed and provided crucial feedback for all the experimental part. W.H.L., L.Y.P., N.B.M.A. and C.H.C. were responsible for the final content check. All the authors have reviewed the manuscript.

Competing Interest

The authors declare no competing interests.

Table 1. IC₅₀ of cationic parent surfactants on 3T3 and MDA-MB-231 cell lines

Cell lines	IC ₅₀ of Cationic Parent Surfactants (μM)			
	TA ₁₂	TA ₁₄	TA ₁₆	TA ₁₈
3T3	27.75 ± 1.42 ^a	14.40 ± 1.12 ^b	7.32 ± 0.28 ^c	6.50 ± 0.48 ^c
MDA-MB-231	22.50 ± 0.91 ^a	9.43 ± 0.46 ^b	6.38 ± 0.32 ^c	5.77 ± 0.25 ^c
Cell lines	IC ₅₀ of Anionic Parent Surfactants (μM)			
	TAC ₁₂	TAC ₁₄	TAC ₁₆	TAC ₁₈
3T3	56.59 ± 0.49 ^a	21.20 ± 0.92 ^b	14.02 ± 0.51 ^c	11.00 ± 0.21 ^d
MDA-MB-231	51.11 ± 2.91 ^a	18.84 ± 1.09 ^b	11.69 ± 0.57 ^c	9.28 ± 0.29 ^c

*Values were means ± standard deviation (n = 3). Values with different letters within the same row were significantly different ($p < 0.05$).

Table 2. IC₅₀ of anionic parent surfactants on 3T3 and MDA-MB-231 cell lines

Cell lines	IC ₅₀ of Anionic Parent Surfactants (μM)					
	C ₈	C ₁₀	C ₁₂	C ₁₄	C ₁₆	C ₁₈
3T3		>500			419.59 ± 3.01 ^a	243.16 ± 1.48 ^b
MDA-MB-231		>500			231.49 ± 5.95 ^a	102.88 ± 1.21 ^b

*Values were means ± standard deviation (n = 3). Values with different letters within the same row were significantly different ($p < 0.05$).

Table 3. IC₅₀ of catanionic surfactants on 3T3 cell line

Anionic Surfactant	IC ₅₀ of Catanionic Surfactants (μM)			
	Cationic Surfactant			
	TA ₁₂	TA ₁₄	TA ₁₆	TA ₁₈
C ₈	43.76 ± 0.92 ^a	19.65 ± 0.72 ^b	10.79 ± 0.64 ^c	9.75 ± 0.27 ^c
C ₁₀	39.60 ± 0.39 ^a	15.34 ± 0.56 ^b	10.20 ± 0.23 ^c	9.20 ± 0.35 ^c
C ₁₂	33.01 ± 0.99 ^a	14.45 ± 0.94 ^b	9.45 ± 0.37 ^c	8.99 ± 0.30 ^c
C ₁₄	29.23 ± 2.08 ^a	12.12 ± 0.43 ^b	8.44 ± 0.29 ^c	7.05 ± 0.35 ^c
C ₁₆	28.20 ± 1.34 ^a	11.30 ± 0.20 ^b	7.54 ± 0.36 ^c	6.56 ± 0.33 ^c
C ₁₈	27.14 ± 1.53 ^a	10.11 ± 0.50 ^b	6.11 ± 0.27 ^c	5.90 ± 0.28 ^c
	TAC ₁₂	TAC ₁₄	TAC ₁₆	TAC ₁₈
C ₈	63.94 ± 1.36 ^a	29.90 ± 0.60 ^b	17.47 ± 0.42 ^c	12.75 ± 0.52 ^d
C ₁₀	62.03 ± 0.47 ^a	26.67 ± 0.50 ^b	16.13 ± 0.22 ^c	12.29 ± 0.07 ^d
C ₁₂	61.21 ± 0.41 ^a	24.93 ± 0.93 ^b	15.25 ± 0.36 ^c	11.96 ± 0.18 ^d
C ₁₄	60.08 ± 0.79 ^a	19.17 ± 0.19 ^b	14.25 ± 0.66 ^c	11.54 ± 0.01 ^d
C ₁₆	59.31 ± 0.98 ^a	18.54 ± 0.36 ^b	13.61 ± 0.20 ^c	11.17 ± 0.19 ^d
C ₁₈	58.25 ± 0.24 ^a	17.37 ± 0.27 ^b	10.70 ± 0.28 ^c	10.40 ± 0.17 ^c

*Values were means ± standard deviation (n = 3). Values with different letters within the same row were significantly different ($p < 0.05$).

Table 4. IC₅₀ of catanionic surfactants on MDA-MB-231 cell line

Anionic Surfactant	IC ₅₀ of Catanionic Surfactants (μM)			
	Cationic Surfactant			
	TA ₁₂	TA ₁₄	TA ₁₆	TA ₁₈
C ₈	29.76 ± 0.95 ^a	18.51 ± 0.29 ^b	8.24 ± 0.45 ^c	7.19 ± 0.25 ^c
C ₁₀	26.90 ± 0.38 ^a	10.84 ± 0.33 ^b	7.29 ± 0.16 ^c	6.69 ± 0.43 ^c
C ₁₂	25.63 ± 0.12 ^a	9.61 ± 0.24 ^b	6.43 ± 0.46 ^c	5.90 ± 0.35 ^c
C ₁₄	23.91 ± 0.50 ^a	9.02 ± 0.25 ^b	6.12 ± 0.33 ^c	5.43 ± 0.11 ^c
C ₁₆	23.17 ± 0.71 ^a	8.32 ± 0.47 ^b	5.65 ± 0.17 ^c	5.09 ± 0.16 ^c
C ₁₈	21.06 ± 0.30 ^a	5.71 ± 0.17 ^b	4.61 ± 0.11 ^c	4.04 ± 0.06 ^d
	TAC ₁₂	TAC ₁₄	TAC ₁₆	TAC ₁₈
C ₈	62.50 ± 0.73 ^a	21.56 ± 1.24 ^b	12.61 ± 0.75 ^c	12.38 ± 0.41 ^c
C ₁₀	60.92 ± 1.65 ^a	20.93 ± 1.27 ^b	12.20 ± 0.30 ^c	12.03 ± 0.55 ^c
C ₁₂	59.73 ± 2.31 ^a	19.93 ± 0.71 ^b	11.73 ± 0.40 ^c	11.44 ± 0.39 ^c
C ₁₄	58.62 ± 1.35 ^a	19.35 ± 0.98 ^b	11.36 ± 0.60 ^c	11.11 ± 0.62 ^c
C ₁₆	57.80 ± 2.15 ^a	18.30 ± 0.34 ^b	11.04 ± 0.63 ^c	10.51 ± 0.63 ^c
C ₁₈	56.92 ± 2.14 ^a	17.30 ± 0.66 ^b	10.28 ± 0.48 ^c	9.86 ± 0.24 ^c

*Values were means ± standard deviation (n = 3). Values with different letters within the same row were significantly different ($p < 0.05$).

Table 5. IC₅₀ of TRF and curcumin on 3T3 and MDA-MB-231 cell line

Cell lines	IC ₅₀ of Anti-Cancer Agents (µg/mL)	
	TRF	Curcumin
3T3	28.39 ± 0.89 ^a	75.80 ± 0.51
MDA-MB-231	22.77 ± 0.34 ^a	42.70 ± 1.08

*Values were means ± standard deviation (n = 3). Values with different letters within the same row were significantly different ($p < 0.05$).

Table 6. Combination Index of different weight ratios of TRF:C₁₈TA₁₈ and curcumin:C₁₈TA₁₈ on 3T3 and MDA-MB-231 cell line

Weight Ratio	Combination Index			
	TRF		Curcumin	
	MDA-MB-231	3T3	MDA-MB-231	3T3
1:1	2.86	5.22	-	-
1:3	2.45	4.43	-	-
1:5	2.15	4.22	3.09	3.58
1:10	1.60	4.10	2.51	2.70
1:20	1.36	3.94	2.41	2.61
1:30	1.21	3.93	2.26	1.79

*The combination index was calculated using Chou and Talalay's Combination Index equation.

Figure 1. IC₅₀ of TA₁₆, TA₁₈, C_nTA₁₆ and C_nTA₁₈ on MDA-MB-231 cell line after 24 hours treatment. The data were expressed as means $\pm$ standard deviation ($n = 3$). Data with different letters within the same group were significantly different ($p < 0.05$) compared with TA_m, respectively.

Figure 2. IC₅₀ of TA₁₆, TA₁₈, C₁₈TA₁₆ and C₁₈TA₁₈ on 3T3 and MDA-MB-231 cell lines after 24 hours treatment. The data were expressed as means $\pm$ standard deviation ($n = 3$). Data with different letters within the same group were significantly different ($p < 0.05$).

(a)

(b)

Figure 3. IC_{50} of TA_{18} , different weight ratios of TRF: $C_{18}TA_{18}$ and curcumin: $C_{18}TA_{18}$ on (a) 3T3 cell line and (b) MDA-MB-231 cell line after 24 hours treatment. The data were expressed as means $\pm$ standard deviation ($n = 3$). Data with different small letters within the same group were significantly different ($p < 0.05$) by comparing with $C_{18}TA_{18}$. Data with different capital letters within the same group were significantly different ($p < 0.05$) between the same anti-cancer agent treatment.

Figures

Figure 1

IC₅₀ of TA₁₆, TA₁₈, CnTA₁₆ and CnTA₁₈ on MDA-MB-231 cell line after 24 hours treatment. The data were expressed as means ± standard deviation (n = 3). Data with different letters within the same group were significantly different (p < 0.05) compared with TAm, respectively.

Figure 2

IC50 of TA16, TA18, C18TA16 and C18TA18 on 3T3 and MDA-MB-231 cell lines after 24 hours treatment. The data were expressed as means $\pm$ standard deviation (n = 3). Data with different letters within the same group were significantly different (p < 0.05).

(a)

(b)

Figure 3

IC50 of TA18, different weight ratios of TRF:C18TA18 and curcumin:C18TA18 on (a) 3T3 cell line and (b) MDA-MB-231 cell line after 24 hours treatment. The data were expressed as means $\pm$ standard deviation (n = 3). Data with different small letters within the same group were significantly different (p < 0.05) by comparing with C18TA18. Data with different capital letters within the same group were significantly different (p < 0.05) between the same anti-cancer agent treatment.