RESEARCH ARTICLE

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Preparation and Characterization of Rutin-Encapsulated Polymeric Micelles and Studies of Synergism with Bioactive Benzoic Acids and Triazolofluoroquinolones as Anticancer Nanomedicines

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Abstract

Background: The study aimed to examine rutin micelles of advanced superlative dual cytotoxicity-antiinflammtion bioefficacies in substantially novel submicro-nanoaffinities vs. both the raw rutin and reference proapoptotic cisplatin. **Methodology:** Antiproliferative capabilities of rutin, benzoic acid (BA) and triazolofluoroqunolone (TFQ) derivatives were reported; hence chemosensitizing effects of rutin or its polymeric micelles (of improved solubility and bioavailability via direct dissolution using the amphiphilic copolymer Pluronic P123) in co-incubations with 5 BAs or 3 TFQ derivatives in a panel of 6 cancer cell lines were verified. **Results:** Rutin loading in micelles was achieved with a loading efficiency of 59.5 ± 2.9%. The particle size of the micelles was found to be 18 ± 2 nm. Though Rutin loaded nanomicelles were of minimal DPPH radical scavenging activity; they had nitrogen oxide (NO) radical scavenging activity in lipopolysaccharide-induced RAW264.7 macrophages with equipotency to indomethacin (IC_{50} values (μM) 73.03 vs. 60.88; p=0.057). Remarkably nano-micelle formulation of rutin was proved of significantly more potent antineoplastic bioactivity with submicro-nanomolar affinities in the 6 cancer cell lines vs. both free rutin's and cisplatin's (except A549 lung cancer cell line). Rrutin nanomicelles chemo-sensitized all selected 8 cotreatments with BA derivatives and TFQs and, thus reducing the dose used against breast cancer MCF7 cells to submicro-nanomolar affinities of greater potencies than cisplatin's. Except for Triazolo-4-anisidine cipro butyl acid in PANC1, 2-Amino-3,5-Di iodo BA in A375 and 4-Nitrophenol in A549 incubations; rutin loaded nanomicelles chemosensitized 7/8 cotreating selected benzoic acid (BAs) derivatives and TFQs and chemosensitized pancreatic PANC1, skin A375 and lung A549 cancer cell lines, thus reducing the dose to submicro-nanomolar affinities of greater potencies than cisplatin's. Rutin loaded nanomicelles chemosensitize 6/8 cotreating selected benzoic acid (BA) derivatives and TFQs (except for 2-Amino-5-Bromo Benzoic Acid and Triazolo-4-anisidine cipro butyl acid), thus reducing the dose used against resistant CACO2 colorectal cancer cells.

Keywords: Rutin- Benzoic acid - Triazolofluoroquinolones-Cisplatin and cancer

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Introduction

Medicinal plants are an essential part of traditional medicine since ancient era and they have been important tools for research and development of new drugs (Ganeshpurkar and Saluja 2017; Caparica 2020). Among them, Flavonoids are one of the most representative classes of plant secondary metabolites that have low molecular weight, occurring throughout the plant kingdom (Lopez-Lazaro 2002; Ren et al., 2003). Rutin is polyphenolic bioflavonoid, broadly extracted from natural sources such as buckwheat, vegetables, apples and black tea (Kreft et al., 1999; Huang et al., 2012). Various studies have found that rutin flavonoids have anticancer activity includes cell proliferation inhibition, a lower reduction glutathione (GSH) and apoptosis stimulation in cancer cells (Elsayed et al., 2017; Vadapalli et al., 2017). However, the poor hydrophilicity of rutin limits its therapeutic activity. Therefore, efforts are required to improve the physicochemical characteristics and anticancer effects of rutin. Thus, in the present study, rutin micelles were prepared using the amphiphilic copolymer Pluronic P123 by the direct dissolution method in order to improve its physicochemical and anticancer properties.

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P123 is a triblock copolymer that consists of poly (ethylene oxide) (PEO) and poly (propylene oxide) (PPO) repeating units. The amphiphilicity of this copolymer leads to the formation of micelles in aqueous solutions, where the hydrophobic core contains PPO blocks and the hydrophilic surface layer is comprised of PEO blocks (Pitto-Barry and Barry 2014). Also benzoic acid and triazolofluoroqunolone derivatives were reported for their antiproliferative properties. Benzoic acid could suppress the growth of cancer cells by angiogenesis and invasion inhibition, and apoptosis induction (Zhao and Hu 2013). Also Triazolofluoroquinolones (TFQs) has significance in medicinal chemistry with diverse biological activities including antinflammatory, anticancer, antioxidant properties (Farokhzad and Langer 2009). Furthermore co-incubation treatments in cancer cell lines panel for BA and TFQ derivatives with rutin or its polymeric micelles can prove definitely promising in cancer chemopreventive/ therapeutic aims.

This rutin polymeric micelles drug does justify and rationalize our aims towards antiproliferativerutin polymeric micelles. Additional rationalization for this research comes from the fact that polymeric micelles technology is superior to traditional pharmaceuticals methods because it improves the safety and efficacy of the drugs and increases patient compliance (Su et al., 2019). Given the emerging evidence for the anti-cancer activity of many flavonoids, and the advantages of nanotechnology in targeted drug delivery; the study aim was to examine rutin micelles of advanced superlative dual cytotoxicity-antiinflammtion bioefficacies in substantially novel submicro-nanoaffinities vs. both the raw rutin and reference proapoptotic cisplatin. Moreover antiproliferative capabilities of rutin, benzoic acid (BA) and triazolofluoroqunolone (TFQ) derivatives were reported; hence chemosensitizing effects of rutin or its polymeric micelles (of improved solubility and bioavailability via direct dissolution using the amphiphilic copolymer Pluronic P123) in co-incubations with 5 BAs or 3 TFQ derivatives in a panel of 6 cancer cell lines were verified . In effect as we propose to design nano-scale polymeric micelles for efficient and targeted delivery of flavonoids to cancer cells. The project will start from the preparation and characterization of the flavonoid-loaded micelles, followed by determination of their combined anti-tumor activity with bioactive benzoic acids and triazolofluoroquinolones in pancreatic PANC1, breast MCF7, colorectal CACO2, skin A375, lung A549 and prostate PC3 cancer cell lines compared to the free compounds and reference drugs. So we hypothesize that these compounds (Table 1) will have antiproliferation properties and selective cytotoxicity vs. previously studied compounds. Moreover Tthis work is basically constructed for elucidation of possible molecular antineoplastic action mechanism via antiinflammation (Haj Hussein et al., 2022; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022).

Materials and Methods

Breast cancer cell line MCF7 (ATCC® HTB-22), A375 human skin cancer cell line (ATCC® CRL-1619), PANC1 pancreatic cell line (ATCC® CRL-1469), A549 lung cancer cell line (ATCC® CCL-185), CACO2 colorectal cancer cell line (ATCC®HTB-37) and PC-3 prostate cancer cell line (ATCC® CRL-1435) were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Foetal Bovine Serum (FBS) (Bio Whittaker, Verviers, Belgium), 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid HEPES Buffer (10 mM), gentamicin (50 µg/mL), L- glutamine (100 µg/mL), streptomycin (100 mg/mL), penicillin (100 µg/mL), (HEPES) Buffer, (Sigma, St. Luis, MO, USA) whereas Sulforhodamine B was from Santa Cruz Biotechnology, Inc. Texas, USA. BA and TFQ derivatives were a gift of Professor Yusuf AL Hiari while the rutin was supplied from local manufactories. The benzoic acid (BA) and triazolofluoroquinolone (TFQ) derivatives used in the research are shown in Table 1.

Preparation and characterization of rutin-loaded polymeric micelles

Micelles were prepared using the amphiphilic copolymer Pluronic P123 (P123, Sigma, St. Louis, MO, USA) by the direct dissolution method. P123 was dissolved in ultrapure water at 5% w/v. Five milligrams of rutin hydrate (Sigma) was weighed in a 20 mL glass vial, to which 10 mL of the P123 solution was added. The mixture was stirred vigorously for 48 h to solubilize rutin. After 48 h, the contents of the vial were centrifuged at 4,000 rpm for 10 min (Hermle Z326K centrifuge, Wehingen, Germany) to precipitate undissolved drug. The supernatant containing rutin-loaded P123 micelles was removed and stored at 4 oC. For the characterization, 100 µL of the micelle solution was diluted with 900 µL DMSO to breakdown the micelles and release rutin. The UV absorbance of the sample was measured at 360 nm (Shimadzu UV-1800 spectrophotometer, Kyoto, Japan). The concentration of rutin in the micelles was calculated based on a calibration curve of rutin absorbance at 360 nm versus concentration in DMSO ($y = 0.0296x - 0.0064$). Rutin loading efficiency was calculated based on the following equation:

Loading efficiency (%) = (Total amount of rutin in micelles / Added amount of rutin) * 100%

Rutin loading efficiency was determined from three different batches of micelles and reported as mean ± SD (standard deviation). In addition, particle size of the micelles was measured by diluting 200 µL of micelles solution with an equal volume of ultrapure water and analyzing the sample using a Nicomp Nano Z3000 instrument (Particle Sizing Systems, Santa Barbara, CA, USA). Measurements were reported as mean intensity diameter ± SD from three different batches of micelles.

DPPH free radical scavenger assay

This method depends on the reduction of the radicals resulting in a color change from oxidized purple to reduced yellow. Principally Diphenyl-2-picryl-hydrazyl (DPPH) undergoes reduction in methanol (MeOH) solution, in the presence of a hydrogen-donating compound due to the formation of the non-radical form DPPH-H. This change in color can be quantitatively measured using a

spectrophotometer at 515–520 nm. In contrast to other radical scavenging assays, a DPPH radical is stable and can provide reproducible spectroscopic values (Haj Hussein et al., 2022; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022). A DPPH solution (0.2 mM) was diluted with MeOH and then mixed with test compounds as well as ascorbic acid with a DPPH solution in a concentration ratio of 1:1 using a 96-well plate (so that a final concentration range 6.25-200 µg/mL was obtained for test agents); the treated solution was incubated one hour isolated from light. Finally, a change in absorbance at 517 nm wavelength was measured using microplate reader (Bio-Tek Instrument, USA). Ascorbic acid was the robust and classical standard radical scavenging reference agent for comparison purposes. The calculation of the DPPH radical scavenging activity inhibition was determined by the following equation where A represents photometric absorbance: in % = (A control – A sample) / A control x 100% (Haj Hussein et al., 2022; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022).

Antiinflammatory (Nitrite) determination in vitro

RAW 264.7 mouse macrophage cell line (ATCC® TIB-71) were cultured in high glucose DMEM supplemented with 10% (FBS), penicillin (100 U/mL), streptomycin (100 µg/mL), and L-glutamate (100 µg/mL) in a 37 oC humidified atmosphere with 95% air and 5% CO2. Confluent macrophages (2 x 105 /well) were incubated with macrophage prompting lipopolysaccharide (LPS; 20 µg/mL; Sigma, St. Luis, MO, USA) added simultaneously with indomethacin (25-200 µg/mL) as the positive control (Arabiyat, et al., 2019; Haj Hussein et al., 2022; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022) and test compounds at different concentrations (5-200 µg/mL, , except for cerivastatin of range: 0.0001-10 µg/mL), for 24 hour incubations. A 100 µL Griess reagent (50 µL of 1 % Sulfanilamide in 5 % phosphoric acid and 50 µL of 0.1 % napthylehtyllenediamine-HCL) were mixed with aliquots of 100 µL of cell culture media and incubated at R.T. for 10 minutes. Absorbance at 550 nm was determined using microplate reader (Biotekmultiwell plate reader MQX200, USA). The concentration of nitrite was determined by comparison with sodium nitrite standard curve. SRB cytotoxity protocol was performed for evaluation of the effect of studied test compounds on RAW 264.7 viability (AbdulFattah, et al., 2019; Arabiyat et al., 2019; Mamdooh et al., 2019; AlKhalil et al., 2020; Hamdan et al., 2020 ; Shakoor et al., 2021; Haj Hussein et al., 2022; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022).

Viability assays for antiproliferative capacities of test compounds: Sulforhodamine B (SRB) assay

The cytotoxicity measurements were determined using Sulforhodamine B (SRB; Santa Cruz Biotechnology, Inc. Texas, USA) colorimetric assay for cytotoxicity screening (using Spectro Scan 80D UV-VIS spectrophotometer (Sedico Ltd., Nicosia, Cyprus). For cytotoxicity screening, the cells were coincubated with selected BAs and TFQs with rutin or rutin loaded nanomicelles at different concentrations (5-200 µg/mL). The cell lines were cultured in high glucose DMEM (Bio Whittaker, Verviers, Belgium) containing 10% FBS, HEPES Buffer (10 mM), L-glutamine (2 mM), gentamicin (50 µg/mL), penicillin (100 U/mL), and streptomycin sulfate (100 mg/mL). As a robust and classical antineoplastic apoptogenic reference agent (El-Hamoly et al., 2017), cisplatin (1-200 µM) was recruited for comparison purposes (Sweidan et al., 2017 ; Alabsi et al., 2018; Arabiyat et al., 2019; Mamdooh et al., 2019; AlKhalil et al., 2020; Hamdan et al., 2020 ; Kasabri et al., 2020; Al-Nuaimi et al., 2021 ; Shakoor et al., 2021; Haj Hussein et al., 2022; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022). The mechanism of reduction of cell viability was adopted so that Dose–response curves were plotted and values were expressed as percentage of control optical density and IC_{50} values 50% inhibitory concentration were estimated by regression analysis (Piazzini et al., 2019). Triplicate assay approach was performed and the calculated anti-proliferative activities were reported as IC_{50} of tested ± SD (n=3). Selectivity index (SI) is the term that describes the safety of tested drugs. It is calculated by dividing IC_{50} value of tested compound on fibroblasts by the least IC_{50} value of the same compound on any specific pathological cell line (Hoffmann, et al., 2011; Sweidan et al., 2017 ; Arabiyat et al., 2019; Mamdooh et al., 2019; AlKhalil et al., 2020; Hamdan et al., 2020 ; Kasabri et al., 2020; Al-Nuaimi et al., 2021 ; Shakoor et al., 2021; Haj Hussein et al., 2022; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022).

Statistical analysis

The values were presented as mean ± SD of 3 independent experiments. Statistical differences between reference agent and different treatment drugs were determined using GraphPad Prism software unpaired t-test (version 5.01 for Windows; GraphPad software, San Diego, CA, USA). Values were considered significantly different if $P< 0.05$ and highly significantly different if $P<0.001$.

Results

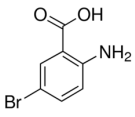
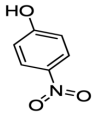
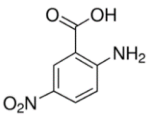
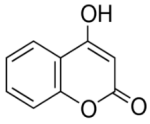
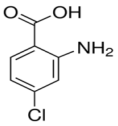
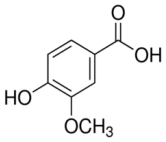
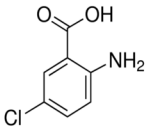
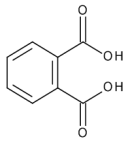
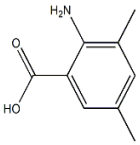
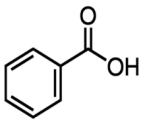
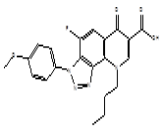
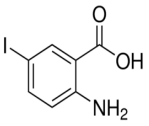
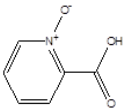
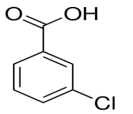
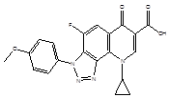
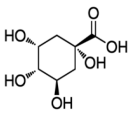
Preparation and characterization of rutin-loaded polymeric micelles

Successful incorporation of rutin in P123 micelles was confirmed by measuring drug loading and particle size. Rutin loading in micelles was achieved with a loading efficiency of 59.5 + 2.9%, producing an aqueous micelle solution equivalent to 474 + 23 µM of rutin. Moreover, the particle size of the micelles was found to be 18 + 2 nm.

DPPH radical scavenging properties of tested benzoic acids, triazolofluoroquinolones (TFQs), rutin, rutin micelles and ascorbic acid

In comparison to the reference agent (Ascorbic acid), most of the tested benzoic acid derivatives and TFQs had appreciable, though inferior to ascorbic acid, radical scavenging capacities in micromolarity range, except for 5-hydroxy anthranilic acid and rutin (Table 2). Furthermore, 5-hydroxy anthranilic acid significantly

Table 1. Benzoic Acid (BA) and Triazolofluoroquinolone (TFQ) Derivatives Used in the Research

Treatment	Structure	Treatment	Structure	Treatment	Structure	Treatment	Structure
2-Amino-5-Bromo Benzoic Acid		4-Nitrophenol		2-Amino-5-Nitro Benzoic Acid		4-Hydroxy coumarin	
2-Amino-4-Chloro Benzoic Acid		Vanillic Acid (4-hydroxy-3-methoxy B.A)		2-Amino-5-Chloro Benzoic Acid		Tert-phthalic Acid	
2-Amino-3,5-Dimethyl Benzoic Acid		Benzoic Acid		Triazolo-4-anisidine cipro butyl acid		2-Amino-5-Iodo Benzoic Acid	
Picolinic acid -N-oxide		m-chloro Benzoic Acid(156.57)		Triazolo-4-anisidine cipro acid		D(-)-Quinic Acid	

(P=0.0008) displayed superior radical scavenging activity, with IC_{50} value 0.209*10-06±1.85*10-07μM in picomolarity range vs. the standard compound (ascorbic acid). The flavonoid rutin with IC_{50} value (1.015±0.06μM) was significantly superior to ascorbic acid effectiveness, unlike its nanocarrier formulation. The rest of the tested compounds TFQs inclusive lacked on comparable potencies.

Anti-inflammatory effects of tested compounds on LPS-prompted RAW264.7 cell line (Table 2)

The inhibitory bioactivities of the compounds against lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW264.7 cell line were analyzed using the Griess assay (Table 2). Compared with the reference agent (indomethacin), 2-Amino-3,5-di iodo benzoic Acid, 5-Hydroxy anthranilic Acid, 4-nitrophenol, rutin nanoparticles and Triazolo-4-anisidine cipro butyl acid had comparable micromolarantiinflammation effectiveness to the classical robust reference agent indomethacin (p>0.05 vs. indomethacin). According to Table 2, the rest of the tested compounds had appreciably reasonable anti-inflammatory effects. Evidently like the rest of benzoic acid derivatives and TFQs; free rutin treatment, unlike its

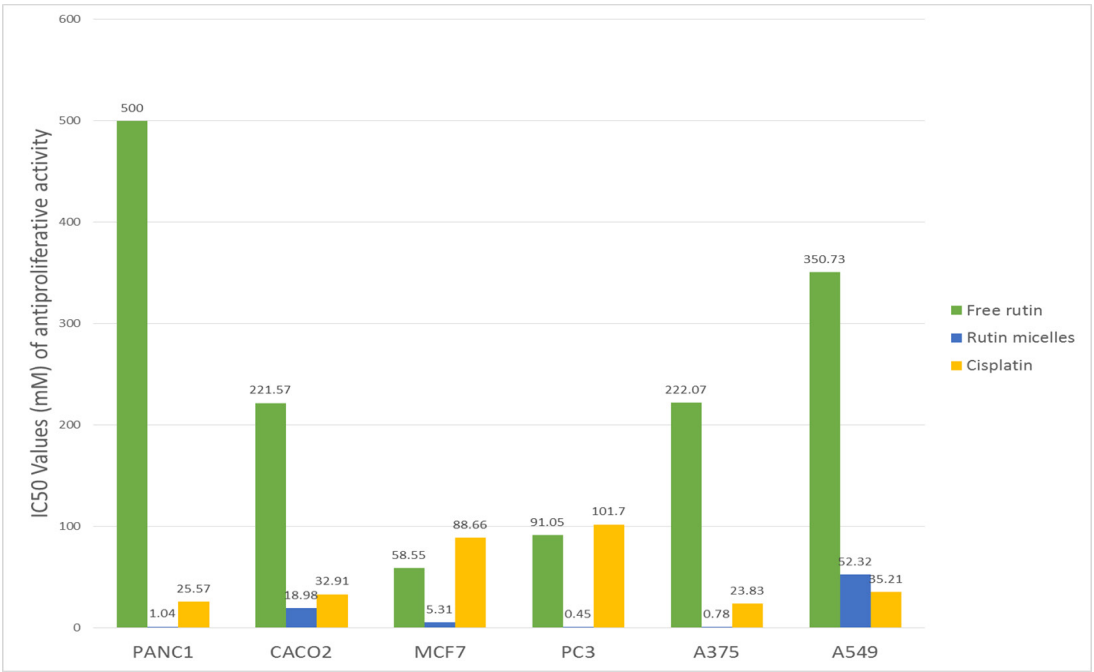


Figure 1. IC_{50} Values (μM) of Antiproliferative Activities of Free Rtuin and Rutin Nanomicelles vs. Cisplatin in the PANC1, CACO2, MCF7, PC3, A375, and A549 cell lines.

Table 2. IC_{50} Values (μM; μg/mL) of *in vitro* DPPH-Radical Scavenging Properties and Antiinflammatory Activities of the Tested Rutin, Its Nanocarrier, Benzoic Acids (BAs) and TFQs vs. respective reference agent vitamin C

Treatment	DPPH radical scavenging IC_{50} value μM (μg/mL)+	NOS- IC_{50} value μM (μg/mL)++
Benzoic acid derivatives		
2-Amino-5-Bromo Benzoic Acid	1889.55±19.24**** (408.2±4.16)	122.21± 3.70** (26.40±0.80)
2-Amino-4-Chloro Benzoic Acid	160.47±25.14*** (27.53±4.31)	155.61±24.71** (26.70±4.24)
2-Amino-3,5-Dimethyl Benzoic Acid	9187.40±1164.65*** (1517.67±192.39)	175.56±21.19** (29.00±3.50)
Picolinic acid -N-oxide	NI	2331.97±103.52**** (324.40±14.40)
3,5-Dichloro anthranilic Acid	NI	101.93±15.05* (21.00±3.10)
4-Hydroxy coumarin	881.13±132.85*** (142.87±21.54)	238.68±23.44*** (38.70±3.80)
Tert-phthalic Acid	NI	740.38±12.64**** (123.00±2.10)
5-Hydroxy anthranilic Acid	**$0.209*10^{-06}$±1.85 *10^{-07}*** ($3.20*10^{-06}$±$2.83*10^{-07}$)**	**62.03±4.57NS (9.50±0.70)**
4-Nitrophenol	NI	**76.20±2.88 NS (10.60±0.40)**
Vanillic Acid (4-hydroxy-3-methoxy B.A)	531.67±13.39**** (89.4±2.25)	430.57±32.11*** (72.40 5.40)
Benzoic Acid	NI	765.64±4.09**** (93.50±0.50)
m-chloro Benzoic Acid	NI	581.11±49.83*** (100.30±8.60)
2-Amino-3,5-Di iodo Benzoic Acid	NI	**48.34±4.89 NS (18.80±1.90)**
2-Amino-5-Iodo Benzoic Acid	5883.99±764.07*** (1547.67±200.97)	141.05±11.41** (37.10±3.00)
D(-)-Quinic Acid	NI	889.84±95.23*** (171.00±18.30)
2-Amino-5-Chloro Benzoic Acid	3464.66±75.37**** (594.467±12.93)	243.04±1.75**** (41.70±0.30)
2-Amino-5-Nitro Benzoic Acid	NI	279.46±28.55*** (50.90±5.20)
TFQ derivatives		
Triazolo-4-hexyl aniline cipro acid	NI	81.9±4.69** (36.73±2.10)
Triazolo-4-anisidine cipro butyl acid	NI	**9.4±1.60*** (3.86±0.66)**
Triazolo-4-anisidine cipro acid	NI	1222.8±183.95*** (482.22±72.54)
Rutin	**1.015±0.06** (0.62±0.04)**	478.73±24.19**** (292.27±14.77)
Rutin micelles	22.31±1.26*** (13.62±0.77)	73.03±4.97NS (44.59±3.03)
Reference Drug	**Ascorbic acid 7.23±1.35 (1.27±0.24)**	**Indomethacin 60.88±6.21 (21.78±2.22)**

Results are mean ± SD (n = 3 independent replicates). IC_{50} values (concentration at which 50% inhibition of DPPH reduction in comparison to non-induced basal incubations) were calculated within testing dose range. P-value is calculated by unpaired t-test between test compound IC_{50} values μM and ascorbic acid IC_{50} values μM (DPPH) using GraphPad Prism software version 8.0.1 * When P<0.05 and ** when P<0.01 or 0.001, *** when P< 0.001or 0.0001, **** when P<0.0001; NS, not significantly different from reference agent; Bolded numerals stand out as the least IC_{50} values (most active) among others. NI, Non-Inhibitory.

nanoformulation, exhibited inferior inhibition to that of indomethacin in LPS-induced inflammation in RAW264.7 macrophages.

Antiproliferative activity of tested compounds in cancer cell lines (Table 3)

Using the SRB assay for antiproliferative activity of 20 tested compounds against cancer PANC1, MCF7 and $CACO_2$ cell lines was demonstrated with respective IC_{50} values (Table 3). Each cell line showed a different response profile to each of the set of tested 17 benzoic acid (BA) derivatives and 3 TFQs. Cisplatinantiproliferative efficacies in all cancer cell lines were further illustrated. We selected 8 compounds that have the most potent activity over 3 cancer PANC1, MCF7 and CACO2 cell lines. The most potent compounds are 2-Amino-5-Bromo Benzoic Acid, 2-Amino-4-Chloro Benzoic Acid, 5-Hydroxy anthranilic Acid, 4-Nitrophenol, 2-Amino-3,5-Diiodo-Benzoic Acid, Triazolo-4-hexyl aniline cipro acid, Triazolo-4-anisidine cipro butyl acid and Triazolo-

Table 3. IC_{50} Value of Cytotoxicity (as of %Control) in µM (µg/mL) of the Tested Compounds vs. Cisplatin

Treatment	PANC-1	CACO2	MCF-7
Benzoic acid derivatives			
2-Amino-5-Bromo Benzoic Acid	218.60±14.99 **** (47.22±3.24)	**46.29±5.24 NS (10.00±1.13)**	**44.67±4.26*** (9.65±0.92)**
2-Amino-4-Chloro Benzoic Acid	272.55±13.79 **** (46.76±2.37)	14704.51±2205.68* (2523.00±378.45)	164.35±7.42*** (28.20±1.27)
2-Amino-3,5-Dimethyl Benzoic Acid	8219.63±306.17 **** (1357.80±50.58)	NI	2027.36±304.10** (334.90±50.24)
Picolinic acid -N-oxide	6173.17±196.72 **** (858.75±27.37)	1759.04±263.86* (244.70±36.71)	2700.02±405.0** (375.60±56.34)
3,5-Dichloro anthranilic Acid	174.57±19.42 *** (35.97±4.00)	1864.29±192.88** (384.10±39.74)	612.77±30.55**** (126.25±6.29)
4-Hydroxycoumarin	1129.27±117.23 **** (183.10±19.01)	NI	980.94±92.89*** (159.05±15.06)
Tert-phthalic Acid	9000.98±972.02 **** (1495.33±161.48)	NI	893.28±133.99** (148.40±22.26)
5-Hydroxy anthranilic Acid	158.62±5.54 **** (24.29±0.85)	230.83±10.62** (35.35±1.63)	125.05±6.93** (19.15±1.06)
4-Nitrophenol	342.41±2.99 **** (47.63±0.42)	507.15±9.66*** (70.55±1.34)	143.27±17.55** (19.93±2.44)
Vanillic Acid (4-hydroxy-3-methoxy B.A)	11497.67±1552.63 *** (1933.33±261.08)	NI	5828.13±984.02** (980.00±165.46)
Benzoic Acid	184.82±5.44 **** (22.57±0.66)	NI	5213.72±847.25** (636.70±103.47)
m-chloro Benzoic Acid	472.19±0.00 **** (81.50±0.00)	NI	1586.91±124.54*** (273.90±21.50)
2-Amino-3,5-Di iodo Benzoic Acid	121.19±16.96 *** (47.13±6.60)	54.31±8.07* (21.12±3.14)	**65.31±0.36*** (25.40±0.14)**
2-Amino-5-Iodo Benzoic Acid	1275.01±152.67*** (335.37±40.16)	2352.20±90.86*** (618.70±23.90)	2371.21±326.36*** (623.70±85.84)
D(-)-Quinic Acid	NI	NI	3010.70±207.25*** (578.57±39.83)
2-Amino-5-Chloro Benzoic Acid	NI	NI	1007.11±146.71** (172.80±25.17)
2-Amino-5-Nitro Benzoic Acid	NI	NI	**75.49±12.03 NS (13.75±2.19)**
Triazolo-4-hexyl aniline cipro acid	220.13±16.46 **** (98.73±7.38)	1978.25±164.40**** (887.25±73.73)	**76.41±14.13 NS (34.27±6.34)**
Triazolo-4-anisidine cipro butyl acid	446.23±70.20 *** (183.14±28.81)	NI	**77.60±7.92 NS (31.85±3.25)**
Triazolo-4-anisidine cipro acid	662.38±85.67 *** (261.22±33.78)	NI	126.70±5.66*** (49.97±2.23)
Reference Drug Cisplatin	**25.57±2.90 (7.67±0.87)**	**32.91±4.49 (9.87±1.35)**	**88.66±1.33 (26.60±0.40)**

Results are mean ± SD (n = 3-4 independent replicates). IC_{50} values (concentration at which 50% inhibition of cell proliferation took place in comparison to non-induced basal 72 h incubations) were calculated within 0.1-400 µg/mL range. NI is a lack of cytotoxicity within the tested 0.1-200 µg/mL concentration range. P-value calculated by unpaired t-test between test compound IC_{50} values and cisplatin's (µM) using Graph Pad Prism software version 8.0.1. * When P<0.05 and ** when P<0.01 or 0.001, *** when P< 0.001or 0.0001, **** when P<0.0001, NS: not significantly different from reference agent. Bolded numerals that stand out as the least IC_{50} values (most active) among others enlisted in the same tested cell line

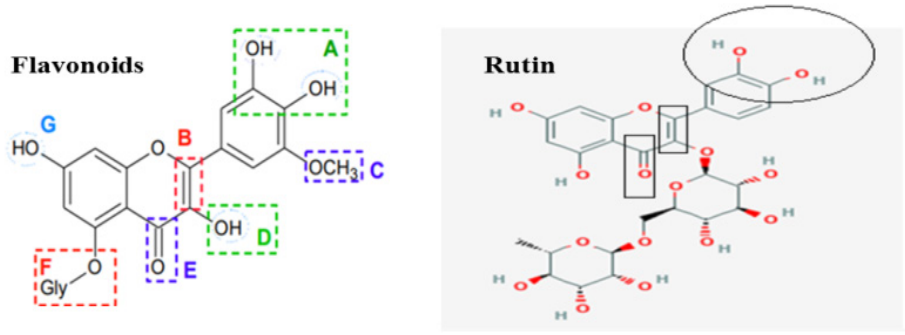


Figure 2. Structure-Activity-Relationship (SAR) for Antiproliferative Activity of Flavonoids and Rutin (Latos-Brozio, and Masek 2019)

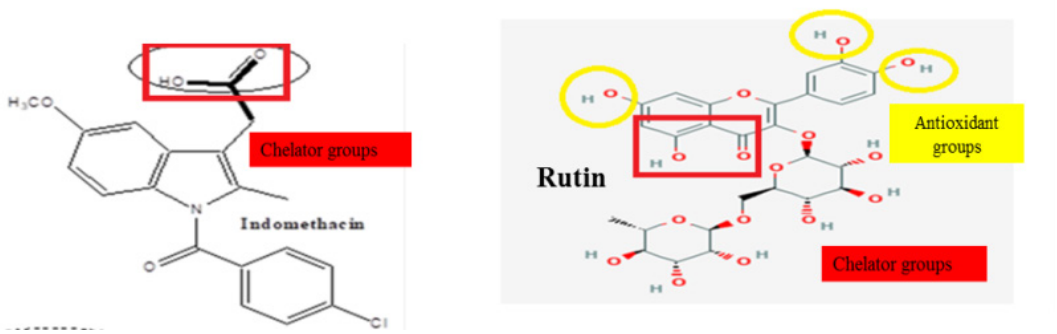


Figure 3. Chemical Classes of Flavonoids and NSAIDs with Antiproliferative, Antiinflammatory Antioxidant Activities (Nimse, and Pal 2015)

4-anisidine cipro acid (Table 3).

Comparisons of antineoplastic bioactivity of selected benzoic acid (BA) derivatives and TFQs to cisplatin's before cotreating with rutin loaded nanomicelles in PANC1, MCF7, CACO2, A375, A549 and PC3 cancer cell lines (Table 4)

In Table 4; the benzoic acid derivative 2-Amino-5-Bromo Benzoic Acid proved equipotency to cisplatin in highly resistant CACO2 and greater potency vs. cisplatin in MCF7 breast cancer cell growth inhibition. All selected 8 compounds lacked antineoplastic bioeffectiveness in PANC1 pancreatic and A549 lung cancer cells. Equally 2-Amino-4-Chloro Benzoic Acid, 5-Hydroxy anthranilic Acid, 4-Nitrophenol, 2-Amino-3,5-Di iodo Benzoic Acid and 3 TFQs were markedly ineffective in CACO2 colorectal cancer cell line (Tables 3 and 4).Exceptionally in MCF7; benzoic acids 2-Amino-5-Bromo Benzoic Acid

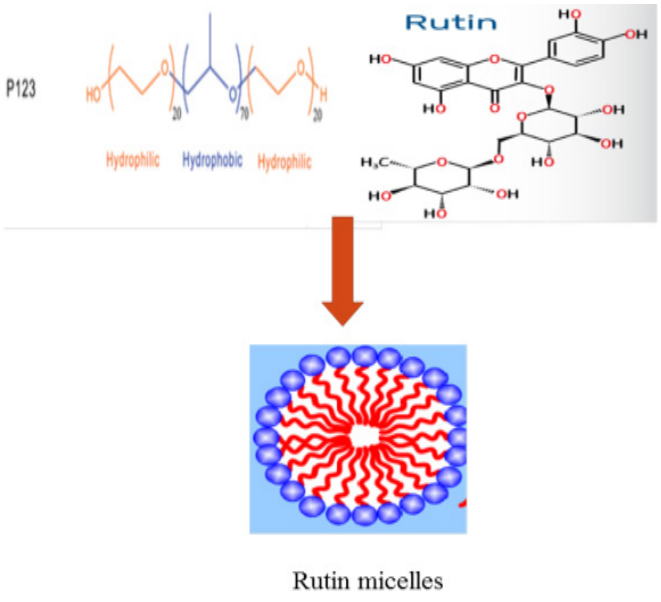


Figure 4. Graphical Illustration of the Preparation of Rutin-Loaded Nanomicelles

Table 4. Cytotoxicity (as of %Control) IC_{50} Value in µM (µg/mL) of Selected Benzoic Acids (BAs), TFQs, Rutin, Rutin Micelles in addition to IC_{50} of the Combined Benzoic Acid (BA) and TFQ with Rutin Free Compound and Rutin Micelles vs. Reference Drug in a Diverse Panel Cancer of Cancer Cell Lines.

			PANC1 cell line			CACO2 cell line	
Benzoic acid derivatives		Benzoic acid (BAs) IC_{50} value in µM (µg/mL)	free rutin IC_{50}NI +cotreatment IC_{50}	Rutin micelles IC_{50} 1.04±0.01*** ΔΔΔ (0.63±0.01) + cotreatment IC_{50}	Benzoic acid (BAs) IC_{50} value in µM (µg/mL)	free rutin IC_{50}221.57±33.97*** (135.27±20.74) +cotreatment IC_{50}	Rutin micelles IC_{50} 18.98±0.58**ΔΔΔ (11.59±0.35) + cotreatment IC_{50}
2-Amino-5-Bromo Benzoic Acid		218.60±14.99**** (47.22±3.24)	NI Ineffective	11.10±1.50** (2.40±0.32) (chemosensitizing)	46.29±5.24 NS (10.00±1.13)	NI Ineffective	328.05±0.64**** (70.87±0.14) Ineffective (antagonistic)
2-Amino-4-Chloro Benzoic Acid		272.55±13.79**** (46.76±2.37)	NI Ineffective	76.17±13.39** (13.07±2.30) (chemosensitizing)	14704.51±2205.68* (2523.00±378.45)	NI Ineffective	423.80±27.90**** (72.72±4.79) (chemosensitizing)
5-Hydroxy anthranilic Acid		158.62±5.54**** (24.29±0.85)	NI Ineffective	59.40±4.53*** (9.10±0.70) (chemosensitizing)	230.83±10.62** (35.35±1.63)	196.87±17.50**** (30.15±2.68) (chemosensitizing)	89.20±13.31** (13.66±2.04) (chemosensitizing)
4-Nitrophenol		342.41±2.99**** (47.63±0.42)	NI Ineffective	2.65±0.07*** (0.37±0.01) (chemosensitizing)	507.15±9.66*** (70.55±1.34)	322.33±26.58**** (44.84±3.70) (chemosensitizing)	39.63±7.53 NS (5.51±1.05) (chemosensitizing)
2-Amino-3,5-Di iodo Benzoic Acid		121.19±16.96*** (47.13±6.60)	NI Ineffective	39.38±7.14* (15.32±2.78) (chemosensitizing)	54.31±8.07* (21.12±3.14)	76.67±11.93**ΔΔΔ (29.82±4.64) Ineffective (antagonistic)	34.90±4.10 NS (13.57±1.59) (chemosensitizing)
TFQs			TFQs IC_{50} value in µM (µg/mL)			TFQs IC_{50} value in µM (µg/mL)	
Triazolo-4-hexyl aniline cipro acid		220.13±16.46**** (98.73±7.38)	NI Ineffective	49.58±4.08** (22.24±1.83) (chemosensitizing)	1978.25±164.40**** (887.25±73.73)	82.30±8.45***ΔΔΔ (36.91±3.79) (chemosensitizing)	129.67±9.12**** (58.16±4.09) (chemosensitizing)
Triazolo-4-anisidine cipro butyl acid		446.23±70.20*** (183.14±28.81)	NI Ineffective	434.88±7.67**** (178.48±3.15) Ineffective	NI	7.57±0.67***ΔΔΔ (3.12±0.27) (maximal chemosensitizing)	1940.00±222.29*** (79.62±91.23) Ineffective
Triazolo-4-anisidine cipro acid		662.38±85.67*** (261.22±33.78)	NI Ineffective	123.91±19.92** (48.87±7.86) (chemosensitizing)	NI	NI Ineffective	82.47±6.85*** (32.52±2.70) (chemosensitizing)
Reference Drug	CISPLATIN		25.57±2.90(7.67±0.87)			32.91±4.49(9.87±1.35)	
			MCF7 cell line			PC3 cell line	
Benzoic acid derivatives		Benzoic acid (BAs) IC_{50} value in µM (µg/mL)	free rutin IC_{50} 58.55±9.05**(35.75±5.53) + cotreatment IC_{50}	Rutinmicelles IC_{50}5.31±0.88****ΔΔΔ (3.24±0.54) + cotreatment IC_{50}	Benzoic acid (BAs) IC_{50} value in µM (µg/mL)	free rutin IC_{50} 91.05±7.00 NS (55.59±4.27) + cotreatment IC_{50}	Rutinmicelles IC_{50}0.45±0.05****ΔΔΔ (0.27±0.03) + cotreatment IC_{50}
2-Amino-5-Bromo Benzoic Acid		44.67±4.26*** (9.65±0.92)	4.44±0.51****ΔΔΔ (0.96±0.11) (chemosensitizing)	4.24±0.23**** (0.92±0.05) (chemosensitizing)	500.15±25.24**** (108.05±5.45)	496.15±78.98*** (107.18±17.06) Ineffective	67.27±8.34** (14.53±1.80) (chemosensitizing)
2-Amino-4-Chloro Benzoic Acid		164.35±7.42*** (28.20±1.27)	30.61±5.65****ΔΔΔ (5.25±0.97) (chemosensitizing)	10.70±1.13**** (1.84±0.19) (chemosensitizing)	NI	NI	213.05±8.41**** (36.56±1.44) (chemosensitizing)
5-Hydroxy anthranilic Acid		125.05±6.93** (19.15±1.06)	23.55±4.34****ΔΔΔ (3.61±0.66) (chemosensitizing)	0.85±0.14**** (0.13±0.02) (chemosensitizing)	306.05±36.11*** (46.87±4.88)	43.67±4.83****ΔΔΔ (6.69±0.74) (chemosensitizing)	99.53±9.69 NS (15.24±1.48) (chemosensitizing)

Table 4. Continued

Reference Drug		CISPLATIN	25.57±2.90(7.67±0.87)			32.91±4.49(9.87±1.35)	
			MCF7 cell line			PC3 cell line	
Benzoic acid derivatives		Benzoic acid (BAs) IC_{50} value in µM (µg/mL)	free rutin IC_{50} 58.55±9.05**(35.75±5.53) + cotreatment IC_{50}	Rutinmicelles IC_{50} 5.31±0.88****ΔΔΔ (3.24±0.54) + cotreatment IC_{50}	Benzoic acid (BAs) IC_{50} value in µM (µg/mL)	free rutin IC_{50} 91.05±7.00 ^{NS} (55.59±4.27) + cotreatment IC_{50}	Rutinmicelles IC_{50} 0.45±0.05****ΔΔΔ (0.27±0.03) + cotreatment IC_{50}
4-Nitrophenol		143.27±17.55** (19.93±2.44)	25.50±1.27****ΔΔΔ (3.55±0.18) (chemosensitizing)	6.66±0.75**** (0.93±0.10) (chemosensitizing)	381.40±33.88*** (53.06±4.71)	124.65±2.47**Δ (17.34±0.34) (chemosensitizing)	897.50±81.32**** (124.85±11.31) Ineffective (antagonistic)
2-Amino-3,5-Di iodo Benzoic Acid		65.31±0.36*** (25.40±0.14)	28.95±4.17****Δ (11.26±1.62) (chemosensitizing)	3.63±0.61**** (1.41±0.24) (chemosensitizing)	18.14±2.52**** (7.06±0.98)	51.41±7.33***Δ (20.00±2.85) Ineffective (antagonistic)	83.12±5.20** (32.33±2.02) Ineffective (antagonistic)
TFQs			TFQs IC_{50} value in µM (µg/mL)			TFQs IC_{50} value in µM (µg/mL)	
Triazolo-4-hexyl aniline cipro acid		76.41±14.13 ^{NS} (34.27±6.34)	40.83±3.38****Δ (18.31±1.52) (chemosensitizing)	0.32±0.06**** (0.14±0.03) (chemosensitizing)	187.57±13.17*** (84.13±5.91)	115.85±16.45 ^{NS} (51.96±7.38) (chemosensitizing)	50.40±4.60*** (22.60±2.06) (chemosensitizing)
Triazolo-4-anisidine cipro butyl acid		77.60±7.92 ^{NS} (31.85±3.25)	46.30±1.41****Δ (19.00±0.58) (chemosensitizing)	0.23±0.04**** (0.09±0.02) (chemosensitizing)	420.53±68.84** (172.59±28.25)	65.13±9.29**Δ (26.73±3.81) (chemosensitizing)	34.15±1.20**** (14.02±0.49) (chemosensitizing)
Triazolo-4-anisidine cipro acid		126.70±5.66*** (49.97±2.23)	19.30±3.37****ΔΔΔ (7.61±1.33) (chemosensitizing)	0.70±0.09**** (0.28±0.04) (chemosensitizing)	804.00±137.23*** (317.07±54.12)	109.70±5.23 ^{NS}Δ (43.26±2.06) (chemosensitizing)	78.57±13.74* (30.98±5.42) (chemosensitizing)
Reference Drug	CISPLATIN		88.66±1.33(26.60±0.40)			101.70±4.10(30.51±1.23)	
Benzoic acid derivatives		Benzoic acid (BAs) IC_{50} value in µM (µg/mL)	free rutin IC_{50} 222.07±40.15** (135.58±24.51) +cotreatment IC_{50}	Rutin micelles IC_{50} 0.78±0.09***ΔΔΔ (0.48±0.05) + cotreatment IC_{50}	Benzoic acid (BAs) IC_{50} value in µM (µg/mL)	free rutin IC_{50} 350.73±15.87****(214.13±9.69) +cotreatment IC_{50}	Rutin micelles IC_{50} 52.23±4.10**ΔΔΔ (31.89±2.50) + cotreatment IC_{50}
2-Amino-5-Bromo Benzoic Acid		29.95±0.07 ^{NS} (6.47±0.02)	22.28±2.22 ^{NS}ΔΔΔ (4.81±0.48) (chemosensitizing)	0.17±0.03*** (0.04±0.01) (chemosensitizing)	415.75±77.43** (89.81±16.73)	3327.33±523.87*** (718.80±113.17) Ineffective (antagonistic)	146.27±3.35**** (31.60±0.72) (chemosensitizing)
2-Amino-4-Chloro Benzoic Acid		17.40±1.41 ^{NS} (2.99±0.24)	22.59±3.53 ^{NS}ΔΔΔ (3.88±0.61) Ineffective	2.83±0.42*** (0.49±0.07) (chemosensitizing)	480.25±5.16**** (82.40±0.89)	NI Ineffective (antagonistic)	234.98±35.31*** (40.32±6.05) (chemosensitizing)
5-Hydroxy anthranilic Acid		44.10±5.80** (6.75±0.89)	35.08±6.06 ^{NS}ΔΔΔ (5.37±0.93) (chemosensitizing)	1.37±0.24*** (0.21±0.04) (chemosensitizing)	345.30±0.28**** (52.88±0.04)	4792.85±431.55**** (733.98±66.09) Ineffective (antagonistic)	62.78±10.82* (9.61±1.66) (chemosensitizing)
4-Nitrophenol		70.50±3.68*** (9.81±0.51)	0.41±0.04***ΔΔΔ (0.06±0.01) (chemosensitizing)	53.11±4.29*** (7.39±0.60) (chemosensitizing)	325.30±3.25**** (45.25±0.45)	319.40±14.73**** (44.43±2.05) Ineffective	1575.40±3.11**** (219.15±0.43) Ineffective (antagonistic)
2-Amino-3,5-Di iodo Benzoic Acid		29.40±4.10 ^{NS} (11.43±1.59)	7.55±1.08**ΔΔΔ (2.94±0.42) (chemosensitizing)	46.06±1.38*** (17.91±0.54) Ineffective (antagonistic)	425.20±32.67**** (165.37±12.71)	219.60±7.73**** (85.41±3.01) chemosensitizing	315.57±42.96*** (122.73±16.71) Chemosensitizing

Table 4. Continued

TFQs	TFQs IC_{50} value in µM (µg/mL)			TFQs IC_{50} value in µM (µg/mL)	
Triazolo-4-hexyl aniline cipro acid	230.50±20.51**** (103.38±9.20)	60.34±11.66**ΔΔΔ (27.06±5.23) (chemosensitizing)	10.70±0.60** (4.80±0.27) (chemosensitizing)	138.47±9.53**** (62.10±4.27)	218.83±8.11**** (98.15±3.64) Ineffective (antagonistic)	103.27±17.12** (46.32±7.68) (chemosensitizing)
Triazolo-4-anisidine cipro butyl acid	493.33±49.56**** (202.47±20.34)	NI Ineffective (antagonistic)	0.03±0.00*** (0.01±0.00) (chemosensitizing)	271.07±37.66*** (111.25±15.46)	763.87±94.83*** (313.50±38.92) Ineffective (antagonistic)	10.53±1.24*** (4.32±0.51) (chemosensitizing)
Triazolo-4-anisidine cipro acid	2092.50±61.52**** (824.68±24.26)	NI Ineffective (antagonistic)	34.07±3.10* (13.44±1.22) (chemosensitizing)	686.10±56.39**** (270.57±22.24)	1383.00±218.13*** (545.40±86.02) Ineffective (antagonistic)	3.22±0.54**** (1.27±0.21) (chemosensitizing)
Reference Drug CISPLATIN	23.83±4.00(7.15±1.20)				35.21±0.03(10.56±0.01)	

Results are mean ± SD (n = 3-4 independent replicates). IC_{50} values (concentration at which 50% inhibition of cell proliferation took place in comparllen to non-induced basal 72 h incubations) were calculated within 0.1-400 µg/mL range. NI is a lack of cytotoxicity within the tested 0.1-200 µg/mL concentration range. P-value calculated by unpaired t-test between test compound IC_{50} values and cisplatin's (µM) using Graph Pad Prism software version 8.0.1. * When P<0.05 and ** when P< 0.001or 0.0001, *** when P<0.0001, NS: not significantly different from reference agent. Bolded numerals that stand out as the least IC_{50} values (most active) among others enlisted in the same tested cell line. Δ When P<0.05 and ΔΔ when P<0.01 or 0.001, ΔΔΔ when P< 0.001or 0.0001, ΔΔΔΔwhen P<0.0001; NS, not significantly different from free rutin

and 2-Amino-3,5-Di iodo Benzoic Acid were exquisitely more potent than cisplatin. 2-Amino-5-Nitro Benzoic Acid and TFQs Triazolo-4-hexyl aniline cipro acid and Triazolo-4-anisidine cipro butyl acid were comparably potent as cisplatin's. 2-Amino-4-Chloro Benzoic Acid, 5-Hydroxy anthranilic Acid and 4-Nitrophenol proved substantially less potent in comparison to cisplatin(Table 4). As of prostate PC3 cancer cell incubations; 2-Amino-3,5-Di iodo Benzoic Acid exerted pronouncedly greater potency vs. cisplatin's. In A375 skin melanoma cells wells (Table 4), 2-Amino-5-Bromo Benzoic Acid, 2-Amino-4-Chloro Benzoic Acid and 2-Amino-3,5-Di iodo Benzoic Acid were comparably potent as cisplatin's.

Comparisons of antineoplastic bioactivity of selected benzoic acid (BA) derivatives and TFQs to cisplatin's after cotreating with rutin loaded nanomicelles in PANC1, MCF7, CACO2, A375, A549 and PC3 cancer cell lines (Table 4)

The modulation of cytotoxicity of promising BA derivatives and TFQs by rutin loaded nanomicelles was further confirmed in Table 4.Remarkably bioflavonoid rutin loaded nanomicelles was proved of significantly more potent antineoplastic bioactivity with submicro-nanomolar affinities in the all 6 cancer cell lines vs. both free rutin's and cisplatin's (except A549 lung cancer cell line) (Table 4and Figure 1).Moreover, in comparison to robust and classical antineoplastic cisplatin; rutin posed equipotency of growth inhibition in PC3 prostate cancer cell line, pronouncedly greater antiproliferation potency in MCF7 breast cancer cell line, but less cytotoxicity effectiveness in resistant CACO2 colorectal cancer cell line, and interestingly lacked similar cell growth suppressing effects in pancreatic PANC1, skin A375 and lung A549 cancer cell lines in vitro (Table 4and Figure 1).

Table 4 displays that rutin loaded nanomicelles synergize with 7/8 cotreating selected benzoic acid (BA) derivatives and TFQs and chemosensitize, thus reducing the dose used against, PANC1 and A549 cells. Triazolo-4-anisidine cipro butyl acid in PANC1 (Table 4) and4- Nitrophenol in A549 were ineffective in wells of rutin loaded nanomicelles wells. Table 4 exhibits that rutin loaded nanomicelles synergize with 6/8 cotreating selected benzoic acid (BA) derivatives and TFQs (except for 2-Amino-5-Bromo Benzoic Acid and Triazolo-4-anisidine cipro butyl acid) and chemosensitize, thus reducing the dose used against resistant CACO2 colorectal cancer cells.

Table 4 demonstrates that rutin loaded nanomicelles synergize with all selected 8 cotreating benzoic acid (BA) derivatives and TFQs and chemosensitize, thus reducing the dose used against, MCF7 breast cancer cells to submicro-nanomolar affinities of greater potencies than cisplatin's. Table 4 illustrates that except for 4-Nitrophenol and 2-Amino-3,5-Di iodo BA; rutin loaded nanomicelles synergize with 6/8 selected cotreating benzoic acid (BA) derivatives and TFQs and chemosensitize, thus reducing the dose used against, PC3 prostate cancer cells to micromolar affinities of greater potencies than cisplatin's. Table 4 shows that except for 2-Amino-3,5-Diiodo BA; rutin loaded nanomicelles synergize with 7/8 selected

cotreating benzoic acid (BA) derivatives and TFQs and chemosensitize, thus reducing the dose used against, skin A375 cancer cells to submicro-nanomolar affinities of greater potencies than cisplatin's.

Discussion

This work aims at preparing and screening rutin micelles against 6 cancer cell lines including Colorectal CACO2, Malignant Melanoma A375; Lung cell line A549; Breast MCF7 cancer ; prostate cancer PC3 and Pancreatic1 (PANC1) cancer cell lines and co-treated with BA or TFQ derivatives .In our study, while free rutin was significantly weaker than indomethacin in terms of antiinflammatory activity, rutin micelles were similar in potency to indomethacin, indicating that solubilization of rutin in a nanocarrier can enhance its antiinflammatory activity in vitro. This may be attributed to increased cellular uptake of rutinnano micelles by the macrophages compared to free rutin. In addition, the flavonoid rutin with IC_{50} value (1.015±0.06 μM) was significantly superior to ascorbic acid effectiveness, unlike its nanocarrier formulation because the concentration of rutin after dissolved from micelles only 5% in first 3 hours, while after 12 hour 60% released, and after 48 hour was 98% released. Furthermore we suggest that rutin micelles when put in MeOH in DPPH for 1 hour it not enough to release all the concentration of rutin in micelles (Nandi et al., 2003).

Various in vivo and in vitro studies have found that rutin flavonoids have anticancer effects through different mechanisms of action, including antiproliferation, angiogenesis inhibition, apoptosis and differentiation induction, antioxidation, carcinogen inactivation, cell cycle arrest, and reversal of multidrug resistance (Nandi et al., 2007). Presence of a carbonyl group at C4, double bond between C2 and C3 are required for cytotoxic activity of rutin and the potency increased with an increasing number of hydroxyl group as shown in Figure 2. Phenol group known that it has anti-oxidant, free radical of scavenging as well as it induces apoptosis by stimulating caspase mediate enzyme these characteristics makes phenolic group works as anticancer activity (Lopez-Lazaro 2002). Rutin has a chelator groups and antioxidant group as shown in Figure 3. This chelator group can bind to divalent and trivalent metals intra and extracellularly. We hypothesize that this chelator group in rutin does interact with DNA-Topoisomerase I complex through a metal, most probably a trivalent metal such iron or copper (Win and Feng 2005). In MCF7 cell line, Triazolo-4-hexyl aniline cipro acid, Triazolo-4-anisidine cipro-butyl acid, Triazolo-4-anisidine cipro acid and 5-Hydroxy anthranilic Acid had the most antiproliferative activity when co-treated with rutin micelles compared to the rest compounds. Moreover, presence of amino and carboxyl groups attached to phenol ring could enhance the antiproliferative activity of these compounds in MCF7 cell line.

P123 was one of most promising Pluronic polymers for targeting and controlling drug and gene delivery. It is interesting to note that P123 is used as pharmaceutical ingredients. Moreover, P123- conjugated polymers have shown a great potential as vectors for drug delivery. The hydroxyl terminal group of PEO-PPO-PEO block copolymer can be activated to couple new functional groups that endow it novel property. When rutin encapsulated with p123 polymer rutin micelles is formed. As shown in Figure 4 the hydrophilic part of P123 will bind with the hydrophilic part of rutin and hydrophobic part will be in the outer core of micelles. The presence of the lipophilic core increases the solubility of poorly water-soluble molecules and offers the possibility to obtain a controlled drug release, while the hydrophilic shell protects the encapsulated drug from the external medium and prevents the interaction with plasma components, resulting in long circulation properties in vivo. Moreover, the small particle size prolongs the residence time in blood circulation, bypassing the liver and spleen filtration and the glomerular elimination, and enhances cellular uptake and the ability to cross epithelial barriers. All these aspects result in increased rutin bioavailability (Latos-Brozio, and Masek 2019).These micelles will facilitate entry of cancer cell line and this was shown in Figure 4. Rutin micelles formulation showed very small particle size (18 nm), which could promote the absorption by enterocytes through endocytosis and help to avoid the uptake by the cells of the reticuloendothelial system and thus bypass the liver and spleen filtration (Nimse, and Pal 2014).

Remarkably bioflavonoid rutin loaded nanomicelles was proved of significantly more potent antineoplastic bioactivity with submicro-nanomolar affinities in the all 6 cancer cell lines vs. both free rutin's and cisplatin's (except A549 lung cancer cell line). In MCF7 cell line, Triazolo-4-hexyl aniline cipro-acid, Triazolo-4-anisidine ciprobutyl acid, Triazolo-4-anisidine cipro acid and 5-Hydroxy anthranilic Acid had the most antiproliferative activity when co-treated with rutin micelles compared to the rest compounds and cisplatin as reference. Moreover, they had IC_{50} less than 50 μM. Furthermore, presence of amino and carboxyl groups attached to phenol ring could enhance the antiproliferative activity of these compounds in MCF7 cell line.

The objective of this study was to design P123 micelles loaded with the poorly soluble anticancer drug rutin. Rutin was well incorporated into P123 micelles with high drug-loading coefficient and encapsulation efficacy. The obtained micelles had a spherical shape with a hydrodynamic diameter of about 18 nm. Our study indicated that rutin loaded polymeric nanomicelles were a novel submicro-nanoagent of rutin with an enhanced antiinflammatory and antiproliferative activity, which could serve as a promising potential candidate for chemotherapy of a diversity of cancers. Cytotoxicity test against PANC1, MCF7, CACO2, A549, A375, PC3 cells showed that rutin-micelles had better in vitro cytotoxicity than rutin free compound. The co-treatment of rutin micelles with benzoic acid or TFQ derivatives had the most synergistic (chemo-sensitizing) growth inhibition in MCF7 compared to other tested cell lines and the polymeric micelles enhance the solubility of rutin and had sustained release activity. Future work includes studying in vivo antiproliferative effect of rutin nanomicelles using animal models of tumorigenesis, studying rutin

nanomicelles in spleen cancer cell line and other cell lines. Clinical testing /toxicity studies of active hits and studying rutin nanomicelles in using other different antioxidant, antiproliferative, and antiinflammatory assays are warranted.

Author Contribution Statement

All authors contributed equally towards rationale conceptualization, experimental design, data collection and analyses, manuscript write up, and proofreading. Authors declare no conflict of interest.

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Availability of Data (if apply to your research)

Data can be made available upon furthering requests to authors

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