

Design, Synthesis and Biological Activities of New Phthalimide and Thiazolidine Derivatives

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Abstract

The combination of pharmacophoric nuclei with different targets has been a strategy for the development of new drugs aimed at improving cancer treatment. A series of ten novel phthalimido-thiazolidine-2,4-dione derivatives were synthesized by two different synthetic routes. The compounds were tested and evaluated *in vitro*, through antineoplastic activities against cancer cells. Cell cycle analyzes and clonogenic assay were also performed. In addition to these tests, *in silico* predictions were performed. The synthesized FT-12 compound (9j) exhibited antiproliferative activity against Panc-1, Sk-mel-28 and PC-3 cells. FT-12 reduced the ability to form new clones, also caused irreversibility in cell cycle, inducing arrest in phase S. Besides, the compound (FT-12) caused necrosis and apoptosis. The results suggest that phthalimido-thiazolidine derivatives may be useful in cancer therapy, highlighting compound FT-12 (9j) as a promising candidate. More studies must be carried out to confirm the viability.

Introduction

Cancer can be defined as a set of diseases that have in common disordered cell growth and invasion of adjacent and non-adjacent tissues and organs [1]. Number of cancer cases continues rising, and much of this is due to the aging and growing population worldwide, along with a growing adoption of behaviors that favor cancer promotion, such as lack of exercise and poor diet. Cancer is the leading cause of death in developed countries, and second cause of death in developing countries, after cardiovascular diseases [2].

Currently available treatments for cancer cells include surgery, chemotherapy, radiotherapy, hormones, and immune therapy. Chemotherapeutic main method of treatment for cancer patients is diffuse systemic chemotherapy using cytostatic agents, which induce apoptosis and or inhibit cell cycle progression [3]. Majority of chemotherapeutic agents do not act exclusively on neoplastic cells, but on dividing cells, causing numerous systemic side effects that represent varying degrees of toxicity depending on factors such as drug exposure time, age, and patient's physical state. Thus, the search for more effective and less toxic drugs for cancer treatment is a constant challenge [4].

Research for new cancer-treatment agents is an important area in medicinal chemistry. Synthesis of new drugs is the combination of distinct pharmacophoric nuclei in a chemical entity, which is termed as molecular hybridization, which is one of the important strategies used by Medicinal Chemistry to obtain more powerful, effective and safer drugs [5]. This strategy aims to link two or more pharmacophoric groups with good biological activities, already reported, creating a new chemical entity with the intention to increase its biological activities to combat various diseases [6,7].

Pharmacophoric group contribute directly to the bioactivity of the molecule and its molecular pathways, directly or indirectly. Those ones with known activity reported in the literature, are good for use in creating new analog compounds [8].

The chemical nucleus of phthalimides (-CO-N (R) -CO-) shows that these structures are hydrophobic, which increases their potential to cross biological membranes *in vivo* [9] (Lamie et al. 2015). Thalidomide is the oldest and most well-known phthalimide derivative. It was initially developed as a sedative hypnotic agent to treat vomiting in pregnancy. Despite its initial successful clinical results, its use has been discontinued because of teratogenic properties. This compound caused severe malformations in the children of women who took the drug during pregnancy [10,11].

Phthalimide pharmacophoric group has been reported in the literature for several biological activities, such as anti-inflammatory activity [12,13], antitumor activity [14,15], analgesic [16], anticonvulsant [17], antituberculosis [18], hypolipemic [19], α -glucosidase inhibitory activity [20], among other activities [11].

Thiazole and thiazolidine nucleus is an important heterocyclic pharmacophore present in several molecules and have significance to create privileged chemical structures possessing pharmacological activities [21]. The analogous structure thiazolidine-2,4-dione is characterized by the formation of new compounds from substitutions or additions in the free portions -NH and -CH₂ of its nucleus [22].

This ring is considered a building block for its versatility. For example, thiazolidinediones are used to treat diabetes, mainly type 2, aiming to decrease hyperglycemia, improving insulin secretion, or reducing insulin resistance in peripheral tissues [23,24]. Many drugs currently available in the market have thiazole or thiazolidine structures, such as thiazofurin and dasatinib (antineoplastic agents), ritonavir (anti-HIV drug), ravuconazole (antifungal agent), nitazoxanide (antiparasitic agent), meloxicam and fentiazac (anti-inflammatory agents), nizatidine (antiulcerogenic agent) and thiametoxam (insecticide) [25].

For example, scaffolds with possible biological activity were developed by linking the thiazole ring to the phthalimide ring, demonstrating the potential of these hybrid structures [26]. In this article we present the synthesis of hybrid compounds, the union of the thiazolidine nucleus with the phthalimide nucleus.

The series of reagents, intermediate products called IPs (**6a-c**), produced for the realization of our final compounds had their methodology approved and with promising results [27,6]. In conclusion, the evaluation of new agents is necessary in order to find more selective and efficient treatments for different types of tumors [28].

In this article we report synthesis and cytostatic activity of novel hybrid compounds phthalimido-thiazolidine-2-4-dione. We examined its efficacies on a panel of human cancer cell lines, and in normal human lymphocytes cells. Finally, with one of our most active compounds (FT-12) (**9j**), we obtained an important *in vitro* activity in tumor growth suppression, also *in silico* analyzes were performed with this compound.

Materials And Methods

Chemical

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Synthesis of new phthalimido-thiazolidine-2,4-dione derivatives (LPSF/ FTs **7a-c** and **9e-k**) were performed by two different methodologies. In Fig. 1 the first synthetic route is shown, in which the final compounds **7a-c** were formed. In Fig. 2, the second synthetic route for the phthalimide and thiazolidine derivatives is shown.

In Fig. 1, the final compounds were synthesized through the following steps: thiazolidine-2,4-dione (**2**) was solubilized with acetonitrile. Separately, NaOH, after maceration, was solubilized in methanol and acetonitrile. Then, the two products are placed to react for 30 minutes and *N*-chloro-methylphthalimide (**1**) was added. The reaction took place at 65 °C of temperature for about 2:30h, accompanied by thin layer chromatography (CCD). After the end of the reaction, the compound formed, the intermediate 3-((1,3-dioxoisindolin-2-yl)methyl)thiazolidine-2,4-dione (LPSF/ FT-01) (**3**), was filtered. The liquid product was placed in the refrigerator, where it stayed for about 72 hours to form white crystals, then filtered. In the second step, LPSF/ IPs (ethyl cyanoacetate esters) (**6a-c**) were synthesized from ethyl cyanoacetate (**4**) with aromatic aldehydes (**5a-c**) using morpholine as catalyst [27]. In the third step, both FT-01 (**3**) and the specific synthesized LPSF/ IPs (**6a-c**) were placed to react in the presence of morpholine (catalyst) at a temperature of 65 °C, forming the final compounds (LPSF / FTs) (**7a-c**). The reaction times varies slightly depending on the IPs reagents. The products were filtered and washed with ethanol.

A different synthesis route is shown in Fig. 2, in this route were synthesized novel thiazophthalimide derivatives by condensation reaction, LPSF/ FTs (**9e-k**). In the second step, the 3-((1,3-dioxoisindolin-2-yl)methyl)thiazolidine-2,4-dione(FT-01) (**3**), compound synthesized in the first step, was placed to react by condensation reaction following the procedures of Harada et al. using specific aldehyde (reagents **8e-k** of Fig. 4) in the presence of ammonium acetate and acetic acid (solvent), at temperature of 110 °C [29] (Harada et al. 2012). At the end of the reaction, the product was filtered and washed with water, generating the final compounds (LPSF / FTs) (**9e-k**). The reaction times varies between 3h and 5h.

Biological

Cell culture. The Cell lines Du-145(prostate cancer), Panc-1(epithelioid carcinoma) and SK-mel-28(malignant melanoma) were cultured in Dulbecco's modified essential medium low glucose (Gibco); MIA-PaCa-2 cells(carcinoma) were cultured in Dulbecco's modified essential medium High glucose (Gibco); Cells HI-60 (acute promyelocytic leukemia) and K562(chronic myelogenous leukemia) were cultured in RPMI 1640 (Gibco); PC-3 cells(adenocarcinoma) were cultured in F12 medium. All of them were supplemented with 10% fetal bovine serum (Gibco), except MIA-PaCa-2 with 2,5% horse serum, 10.000 U of penicillin and 10.000 of streptomycin (Gibco) and were maintained in a humidified atmosphere containing 5% CO₂. The Cells were obtained from the Rio de Janeiro cell bank (BCRJ).

Cytotoxicity assays. The *in vitro* cytotoxicity test was performed on neoplastic cells and peripheral blood mononuclear cells (PBMCs) with different concentrations. Cellular cytotoxicity was quantified by the colorimetric method 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) [30].

PBMCs were plated 5x10⁵ cells per well and neoplastic cells were distributed into 96 well plates, cells

concentration was according to the NIH recommendations, the number of cells per well/100µl. After 24h, different concentrations of phthalimido-thiazolidine derivatives (1, 10, 50, and 100 µM) in plates were incubated in a humidified atmosphere at 37°C and 5% CO₂ for 48h (PBMCs) for 72h. After the incubation period, 20 µL of the 5 mg/mL MTT solution diluted in PBS was added; the plates were then protected from light, and were incubated once more in a humidified atmosphere at 37°C and 5% CO₂ for a period of 3h. Then it was added 130 µl of 20% SDS per well, and measured optical density were realized after 24 hours at the 560 nm wavelength. The reading was performed on a microplate reader (EL808 - Biotek®). Determination of the cytotoxic potential of the tested compounds was calculated in relation to the control, which was treated with the 0,1% DMSO vehicle. To determine the concentration of the compound, required for 50% inhibition in vitro (IC₅₀), three independent MTT assays were performed. Doxorubicin (Sigma-Aldrich Co., St. Louis, MO, USA) was used as a positive control.

Cell cycle analysis. Cells were plated in 6-well plates at a concentration of 5×10^5 cells per well and treated at IC₅₀ concentration. After 48 hours, the cells were trypsinized, washed twice with PBS, and fixed in 70% alcohol at 4–6°C; then, the cells were incubated for 24 hours at -20°C. In the next step, the cells were incubated with propidium iodide (5 mg/mL)/RNase A (0.25 mg / mL) in PBS for 30 minutes on ice and were protected from light. Cellular DNA content was quantified by flow cytometry using the Accuri C6 flow cytometer, wherein the percentage of DNA in each phase of the cycle was obtained by the C6 software (Becton, Dickinson and Company, USA).

In vitro scratch assay. Cells were plated in 24-well plates at a density of 1×10^5 , and incubated at 37°C under 5% CO₂ atmosphere for 24 hours. After reaching the necessary confluence, the growth medium was aspirated, and two washes with 1X PBS were performed; then, two perpendicular marking lines (*striae*) were made using a P200 pipette. After the labeling procedure, the cells were treated with complete medium (control), 0.1% DMSO, and FT-12 synthesized, at concentrations previously determined by IC₅₀. They were photographed under the inverted microscope (eclipse Ts2 Nikon) (t = 0) and incubated for 24 hours (t = 0, t = 6, t = 12 and t = 24) under the same conditions as above. Migration assay data were obtained from image analysis through the Image J program (Version 1.49; NIH, USA).

Clonogenic Assay. Cells were initially plated in 24-well cluster plates at a density of 3.0×10^4 cells/well and incubated at 37°C in atmosphere of 5% CO₂. After 24h and 48h, the medium was changed, the cells were treated with 0.1% DMSO (control group), or with the phthalimido-thiazolidine derivative FT-12 (**9j**) at concentration previously determined by the IC₅₀. After 14 days, the colonies were fixed with paraformaldehyde and stained using Methyl Violet (Thermo Fisher Scientific, Waltham, MA, USA). The stained colonies were counted manually.

Statistical analysis. Three independent experiments were performed in triplicate. IC₅₀ values and 95% confidence intervals were obtained with nonlinear regression with the OriginPro program (8.0; OriginLab, Northampton, Massachusetts, USA). Statistical significance was tested with two-tailed unpaired student's *t*-tests in relation to the untreated or positive controls, and differences were considered significant when *p*

values were less than 0.05. Values were expressed as the mean \pm SD of three or more replicate experiments.

In silico predictions. First, we performed computational analysis using PASS online for predicting FT-12 (**9j**) effects and mechanisms of action. PASS online is a software for evaluating the biological potential of compounds and gives a score for probability to be active (Pa) and inactive (Pi). FT-12 was estimated as biological active for a specific activity when $P_a > P_i$ and $P_a > 0.1$ to be considered significant. Genes directly related to FT-12 predicted functions were investigated through ENRICH for gene ontology enrichment analysis of associated biological processes (BP) and KEGG pathways.

Results

Synthesis

The structures of new compounds LPSF/FTs synthesized were confirmed by spectroscopic techniques such as nuclear magnetic resonance (^1H NMR) model Varian 300 or 400 MHz, using solvent $\text{DMSO-}d_6$ and CDCl_3 . The peaks of the RMN signals were designated s - singlet; d - doublet; t - triplet; m - multiplet. Melting points were determined in a capillary tube using Buchi melting point M-565. Infrared spectra (IR) were recorded on a Prestige-21, Shimadzu model 01801. For Mass Spectra (MS), Bruker Daltonics, modelo autoflex III smart beam was utilized.

For example, the final compounds were proven by the presence of the CH_2 group (singlet) attached to the nitrogen atom of thiazolidine in the range of 5.6 ppm in the nuclear magnetic resonance spectrum. In the compound **9k** some hydrogens in the aromatic ring of the radical, with the presence of a nitrogen, were less protected, appearing in the lower field of the spectrum than the hydrogens present in the aromatic phthalimidic ring.

In vitro cytotoxic activity in tumor cells and clonogenic assays

Initially, the eleven compounds synthesized were tested against peripheral blood mononuclear cells (PBMCs). All the synthesized compounds were assessed for *in vitro* cytotoxic effect in PBMCs using MTT assay, as a primary step for investigating their cytotoxic activity. It was observed that phthalimido-thiazolidine derivatives were nontoxic ($\text{IC}_{50} > 100 \mu\text{M}$) in this type of cells. The synthesized compounds were tested in different hematopoietic cells lines and solid tumors, only FT-9 (**9i**) and FT-12 (**9j**) showed antineoplastic activity as shown in Table 1. The compound FT-9 shows activity in two tumor lines (K-562 and Molt-4), while FT-12 in three (PC-3, Panc-1, SK-mel-28).

Table 1
IC₅₀ values of phthalimido-thiazolidine-2-4-dione derivatives in cancer cells

| Compounds | Du-145 | PC-3 | Panc-1 | SK-mel-28 | Miapaca-2 | HL-60 | K-562 | Molt-4 |
|-------------|-----------|--------------|--------------|--------------|-----------|-----------|--------------|--------------|
| FT-2 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 |
| FT-3 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 |
| FT-4 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 |
| FT-6 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 |
| FT-7 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 |
| FT-8 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 |
| FT-9 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | 59.11 ± 4.48 | 64.42 ± 6.36 |
| FT-12 | > 100 | 71.92 ± 1.31 | 31.54 ± 1.33 | 46.64 ± 5.85 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 |
| FT-15 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 | > 100 ± 0 |
| Doxorubicin | 2.2 | 3.4 | 6.8 | 6.36 | > 1 | 0.02 | 6.52 | 5.5 |

To evaluate the cytotoxic potential, the highest and lowest IC₅₀ concentrations were chosen. Initially the inhibition of clone formation was evaluated at 24 and 48 hours of treatment. Results in Fig. 3 showed that the number of viable PC-3 cell colonies was significantly decreased after FT-12 (9j) treatment. Both in the 24-hour (p = 0,0153) and 48-hour (p = 0,0644), there was a reduction in the formation of new clones.

We also evaluated the ability to inhibit the formation of colonies in Panc-1 cells (Fig. 4). Both, in the 24-hour (p = 0,0372) and 48-hour (p = 0,096), there was a reduction in the formation of new clones. Against PC-3 strain, the FT-12 derivative exhibited a greater inhibitory effect with less treatment time. However, against the Panc-1 strain, the inhibitory effect is observed in both treatment times.

Effects of LPSF/FT-12-9j on the cell cycle and on the induction of apoptosis

The FT-12 derivative effects on cell cycle in Panc-1 cells was evaluated. This derivative did not alter the cell cycle progression significantly (Table 2).

Table 2
Effects of phthalimido-thiazolidine-2-4-dione derivative (FT-12) on cell cycle progression of Panc-1 cells after 48h of treatment.

| | Sub-G0 | G0/G1 | S | G2/M |
|-------------|---------------|---------------|-------------|--------------|
| NT | 1.20 ± 0.3 | 49.53 ± 6.5 | 10.37 ± 5.4 | 38.57 ± 5.0 |
| FT-12 | 1.87 ± 0.6 | 43.50 ± 7.9 | 14.57 ± 1.8 | 38.17 ± 6.4 |
| Doxorubicin | 5.53** ± 1.1 | 21.87** ± 3.7 | 17.10 ± 4.0 | 53.37* ± 4.1 |

To evaluate if FT-12 (**9j**) derivative induces other cells to death processes, we tested for analysis by 7-AAD (permeabilidade de membrana marker) and annexin V (apoptosis). The derivative induced a significant increase in necrosis ($p = 0.0202$), also induced by doxorubin treatment ($p = 0.0016$; Fig. 5). Furthermore, FT-12 treatment resulted in a significant increase in apoptosis ($p = 0.0017$).

To investigate apoptosis molecular pathway, we next evaluated the expression of the PARP, cell apoptosis-related protein Poly (ADP-ribose) polymerase in PANC-1 cells. FT-12 treatment did not induce a significant increase (17.35%) in cleaving PARP compared to untreated cells (2.35%), as shown in Fig. 6.

Bioinformatics studies

We chose the most promising synthesized compound to run some *in silico* tests. Through PASS online screening was performed. Some genes (shown in Table 3) were predicted to be targeted by FT-12 in some way (e.g Mcl-1 receptor antagonist).

Table 3
Genes targets: *in silico* predictions of the FT-12 derivative.

| Pa* | Pi** | Gene Symbol | Activity |
|--|-------|-------------|--|
| 0.758 | 0.016 | C5AR1 | Anaphylatoxin receptor antagonist |
| 0.458 | 0.004 | DUSP1 | Dual specificity phosphatase inhibitor |
| 0.43 | 0.01 | CAD | Dihydroorotase inhibitor |
| 0.404 | 0.013 | MCL1 | Mcl-1 antagonist |
| 0.403 | 0.01 | APP | Amyloid beta precursor protein antagonist |
| 0.359 | 0.069 | CYP2C19 | CYP2C19 inducer |
| 0.352 | 0.059 | MYC | Myc inhibitor |
| 0.347 | 0.062 | CFTR | CF transmembrane conductance regulator agonist |
| 0.338 | 0.002 | PIM2 | Pim-2 kinase inhibitor |
| 0.329 | 0.004 | PIM1 | Pim kinase inhibitor |
| 0.326 | 0.054 | GYS1 | Glycogen synthase stimulant |
| 0.309 | 0.003 | PTPRB | Protein-tyrosine phosphatase beta inhibitor |
| 0.302 | 0.079 | PTK2 | Focal adhesion kinase 2 inhibitor |
| 0.281 | 0.004 | BCL2 | Bcl2 antagonist |
| 0.261 | 0.002 | ENPP2 | Autotaxin inhibitor |
| 0.23 | 0.085 | NISCH | Imidazoline I1 receptor agonist |
| 0.212 | 0.005 | IL12 | Interleukin 12 agonist |
| 0.205 | 0.022 | CDA | Cytidine deaminase inhibitor |
| 0.199 | 0.012 | CHRNA3 | Nicotinic alpha3beta4 receptor agonist |
| 0.195 | 0.028 | PTGS1 | Cyclooxygenase 1 inhibitor |
| *Pa (probability "to be active"); **Pi (probability "to be inactive") | | | |

Discussion

The new intermediate obtained FT-01 (**3**), since the first reactions presented satisfactory yields, between 62–81%. The synthesis is also relatively fast, two hours of reaction. Therefore, it was made on a larger scale of production, also obtaining good yields. The main difficulty is the fact that FT-1 (**3**) is not very soluble, making it difficult to use as a reagent.

Seven compounds (FT-2, FT-3, FT-4, FT-6, FT-9, FT-12, FT-15), **9e-k**, were obtained by the condensation methodology [29]. This second methodology (Fig. 2) was used due to the difficulty of synthesis by the first methodology. For the first methodology is necessary to synthesize one more intermediate step (the IPs) to reach the final compound, what makes this synthesis more laborious and costly (Fig. 1). The compounds FT-7, FT-8 and FT-18, **7a-c**, were synthesized by the first and more expensive methodology, following similar steps carried out previously [27]. Research done on the *SciFinder* website, did not present in the literature compounds with similarity sufficient for a good comparison with the obtained thiazophthalimides.

Most of the final reactions did not take much time to be completely finished, the times varied between 1h and 5h. All the final compounds FTs (**7a-c** and **9e-k**) had yields varying between 22.5% -95%, this variation demonstrates that the aldehydes, intermediates of the reaction, have a great influence on the reactivity for the formation of new compounds.

None of the phthalimido-thiazolidine-2-4-dione derivatives (**7a-c**, **9e-k**) showed cytotoxic activity in normal healthy donor cells (PBMCs). It is an important attribute of anti-cancer drugs, be capable of not provoke damaging effects on normal cells. The derivative FT-12, 5-((2-chloro-6-methylquinolin-3-yl)methylene)-3-((1,3-dioxoisindolin-2-yl)methyl)thiazolidine-2,4-dione (**9j**), showed larger spectrum of antineoplastic action with IC_{50} between 31.54 μ M in Panc-1 cells and 71.94 μ M in PC-3 cells. The derivative FT-9 (**9i**) also showed antineoplastic activity with IC_{50} between 59.11 μ M in K-562 cells and 64.42 μ M in Molt-4 cells, both of leukemic origin. The derivative FT-12 showed activity against solid tumor neoplastic cells, whereas the derivative FT-9 showed activity only against hematopoietic cells (Fig. 7).

The FT-12 derivative owns a group quinoline. Quinine and camptothecin are important quinoline alkaloids with important drugs antimalarial and anticancer. Many molecules with a broad range of bioactivities, including antitumor, antimalarial, antibacterial and antifungal, antiparasitic and insecticidal, antiviral, antiplatelet, anti-inflammatory, herbicidal, antioxidant and other activities were studied [31]. Our results demonstrated that the 2-chloro-quinoline ring system had an antiproliferative activity more prominent than others substitutes. Pun et al. evaluated a novel quinoline effect in proliferation of esophageal cancer model *in vivo*, they showed that this compound reduced the tumor size in nude mice xenograft [32]. Mphahlele et al. synthesized new thienoquinolines, which showed cytotoxic effects against human breast adenocarcinoma cell line (MCF-7 cells) with IC_{50} between 0.014 and 1.84 mg/ml [33]. Magar et al. showed chlorine group at the *meta* or *para*-position of the 2-phenyl ring, displayed highly potent topoisomerase II inhibitory activity [34].

We performed analysis to identify specific biological processes that could be affected by the compound FT-12. This included regulation of cell death, regulation of apoptosis, DNA intercalator, programmed cell death, and signal transduction. FT-12 (**9j**) induced a decrease in the formation of clones in both Panc-1 and PC-3. This assay can be used to determine the cytotoxic effects of various treatments, including

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curcumin derivatives exerted inhibitory effects of the migration capacity of PC-3 and Du-145 cells, the clonogenic inhibition effect with decrease in colony formation reached up to 95% following K3F21 treatment at 5 and 10 μM in PC-3 and DU-145 cells [36].

We decided to evaluate the cell death mechanism induced by FT-12 compound in cell Panc-1. The FT-12 derivative induced necrosis and apoptosis, besides inducing cleavage of PARP. Rohitkumar et al. showed that thieno(2,3-b)quinoline (BPTQ) derivatives induced arrest cell cycle in phase S and cleaved Parp in MOLT-4 cells leukemia [37]. Santos et al. showed 1,3-thiazole cause irreversible cancer cell damage by inducing necrosis and apoptosis in HT-29 cancer cells [38].

Conclusion

In summary, synthesized compounds derived from phthalimide and thiazolidine were made by two different synthetic routes, some of them induced cytotoxic activity against tumor cells. The synthesized compound FT-12 (**9j**) also had clonogenic inhibition effect, decreasing colony formation and inhibiting migration in prostate and pancreatic cells. FT-12 (**9j**) derivative also induced necrosis/apoptosis in pancreatic cells. *In silico* evaluation demonstrated that phthalimide-thiazolidine nucleus has the potential to act in several therapeutic targets. More studies must be carried out to confirm the viability of the new compounds.

Experimental

Structural characterization of synthesized compounds: 3-((1,3-dioxisoindolin-2-yl)methyl)thiazolidine-2,4-dione (LPSF/FT-01) (**3**)

$\text{C}_{12}\text{H}_8\text{N}_2\text{O}_4\text{S}$. White solid. Melting point (m.p.): 172°C . Yield: 67 %. IR (KBr, cm^{-1}): 3005 (C-H), 1730 (C = O), 1676 (C = O), 1315 (C-N). ^1H NMR (300 MHz, DMSO-d_6) δ 7.88 (m, 4H, $J = 20.7$ Hz, ArH), 5.33 (s, 2H, - CH_2), 4.19 (s, 2H, - CH_2). ^{13}C -NMR (300 MHz, DMSO-d_6): δ 171.1 (C = O), 170.8, 166.3, 134.8, 123.4, 43.5 (CH_2), 33.5. MS m/z (%): 275.95, calculated 276.02.

5-(4-(1H-imidazol-1-yl)benzylidene)-3-((1,3-dioxisoindolin-2-yl)methyl)thiazolidine-2,4-dione (LPSF/FT-07) (**7a**)

$\text{C}_{22}\text{H}_{14}\text{N}_4\text{O}_4\text{S}$. Yellow solid. m.p.: 263°C . Yield: 44 %. IR (KBr, cm^{-1}): 3136 (C-H), 1732 (C = O), 1598 (C = C), 1320 (C-N), 724 (C-H). ^1H NMR (300 MHz, DMSO-d_6): δ 8,39 (s, 1H, -CH), 7,89 (m, 8H, $J = 42,3$ Hz, ArH), 7,74 (d, 2H, $J = 8,1$ Hz, ArH), 7,14 (s, 1H, ArH), 5,51 (s, 2H, - CH_2). ^{13}C -NMR (300 MHz, DMSO-d_6): δ 166.3, 166.0, 164.4, 138.0, 135.6, 134.8, 132.3, 131.7, 131.2, 131.0, 130.3, 123.4, 120.5, 117.6, 43.8 (CH_2). MS m/z (%): (M + H) $^+$ 431.03, calculated 430.07.

3-((4-((3-((1,3-dioxisoindolin-2-yl)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenyl)

Loading [MathJax]/jax/output/CommonHTML/jax.js (**7b**)

$C_{23}H_{18}N_4O_4S$. Orange solid. m.p.: 241°C. Yield: 61 %. IR (KBr, cm^{-1}): 2917 (C-H), 2248 (-CN), 1721 (C = O), 1582 (C = C), 1319 (C-N). H^1 NMR (300 MHz, DMSO- d_6): δ 7,88 (m, 5H, $J = 34,2$ Hz, FtH), 7,45 (d, 2H, $J = 9,6$ Hz, ArH), 6,91 (d, 2H, $J = 9,3$ Hz, ArH), 5,48 (s, 2H, $-CH_2$), 3,77 (t, 2H, $J = 13,5$ Hz, CH_2), 3,04 (s, 3H, CH_3), 2,76 (t, 2H, $J = 12,9$ Hz, CH_2). ^{13}C -NMR (300 MHz, DMSO- d_6): δ 170.8, 166.3, 166.2, 164.6, 149.97, 134.8, 134.2, 132.4, 131.1, 123.3, 120.4, 112.3, 47.1, 43.5 (CH_2), 33.4, 14.9. MS m/z (%): 446.07, calculated 446.1.

5-(3-bromobenzylidene)-3-((1,3-dioxisoindolin-2-yl)methyl)thiazolidine-2,4-dione (LPSF/FT-18) (**7c**)

$C_{19}H_{11}BrN_2O_4S$. White solid. m.p.: 210°C. Yield: 68 %. IR (KBr, cm^{-1}): 3088 (C-H), 1748 (C = O), 1602 (C = C), 1313 (C-N), 723 (C-H). H^1 NMR (400 MHz, $CDCl_3$): δ 7,89 (m, 2H, $J = 8,4$ Hz, ArH), 7,82 (s, 1H, CH), 7,75 (m, 2H, $J = 8$ Hz, ArH), 7,61 (t, 1H, $J = 3,6$ Hz, ArH), 7,55 (dt, 1H, $J = 11,2$ Hz, ArH), 7,41 (d, 1H, $J = 8,4$ Hz, ArH), 7,34 (t, 1H, $J = 16$ Hz, ArH), 5,67 (s, 2H, CH_2). ^{13}C -NMR (400 MHz, $CDCl_3$): δ 166.5, 164.7, 135.0, 134.5, 133.5, 132.9, 131.5, 130.6, 128.3, 123.9, 123.3, 122.3, 43.5 (CH_2). MS m/z (%): (M + H) $^+$ 442.9, calculated 441.96.

4-((3-((1,3-dioxisoindolin-2-yl)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenyl acetate (LPSF/AA-FT-02) (**9e**)

$C_{21}H_{14}N_2O_6S$. Yellow solid. m.p.: 263°C. Yield: 56,7 %. IR (KBr, cm^{-1}): 1745 (C = O), 1722 (C = O), 1585 (C = C), 725 (C-H). H^1 NMR (300 MHz, DMSO- d_6): δ 7,87 (m, 5H, $J = 28,2$ Hz, CH, FtH), 7,46 (d, 2H, $J = 8,4$ Hz, ArH), 6,91 (d, 2H, $J = 9$ Hz, ArH), 5,49 (s, 2H, $-CH_2$), 2,29 (s, 3H, $-CH_3$). ^{13}C -NMR (300 MHz, DMSO- d_6): δ 166.43, 166.36, 164.62, 160.27, 134.81, 133.94, 132.65, 131.18, 123.69, 123.41, 116.41, 115.87, 43.77. MS m/z (%): (M + H) $^+$ 423.9, calculated 422.05.

N-(4-((3-((1,3-dioxisoindolin-2-yl)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenyl)acetamide (LPSF/FT-03) (**9f**)

$C_{21}H_{15}N_3O_5S$. Yellow solid. m.p.: 277°C. Yield: 80.4 %. IR (KBr, cm^{-1}): 3382 (N-H), 1745 (C = O), 1717 (C = O), 1580 (C = C), 726 (C-H). H^1 NMR (300 MHz, DMSO- d_6): δ 7,88 (m, 5H, $J = 24,3$ Hz, CH, FtH), 7,74 (d, 2H, $J = 8,7$ Hz, ArH), 7,55 (d, 2H, $J = 9$ Hz, ArH), 5,49 (s, 2H, $-CH_2$), 2,07 (s, 3H, $-CH_3$). ^{13}C -NMR (300 MHz, DMSO- d_6): δ 168.8, 166.3, 164.5, 141.5, 134.8, 133.2, 131.3, 131.2, 127.1, 123.4, 119.1, 118.0, 43.7 (CH_2), 40.3, 24.1. MS m/z (%): (M + H) $^+$ 422.02, calculated 421.07.

5-((2,3-dihydrobenzofuran-6-yl)methylene)-3-((1,3-dioxisoindolin-2-yl)methyl)thiazolidine-2,4-dione (LPSF/FT-04) (**9g**)

$C_{21}H_{14}N_2O_5S$. Yellow solid. m.p.: 255.9°C. Yield: 75,5 %. IR (KBr, cm^{-1}): 1779 (C = O), 1736 (C = O), 1594 (C = C), 710 (C-H). H^1 NMR (300 MHz, $CDCl_3$): δ 7,88 (m, 3H, $J = 12,3$ Hz, CH, FtH), 7,74 (m, 2H, $J = 8,7$ Hz, ArH), 7,55 (d, 2H, $J = 9$ Hz, ArH), 5,66 (s, 2H, $-CH_2$), 4,66 (t, 2H, $J = 17,7$ Hz),

3,26 (t, 2H, $J = 17,7$ Hz). ^{13}C -NMR (300 MHz, CDCl_3): δ 166.5, 162.6, 135.2, 134.4, 132.4, 131.5, 128.7, 127.0, 125.7, 123.8, 116.4, 110.2, 72.1, 43.3 (CH_2), 29.1 (CH_3). MS m/z (%): 406.06, calculated 406.11.

3-((1,3-dioxoisindolin-2-yl)methyl)-5-(4-(diphenylamino)benzylidene)thiazolidine-2,4-dione (LPSF/FT-06) (**9h**)

$\text{C}_{31}\text{H}_{21}\text{N}_3\text{O}_4\text{S}$. Yellow solid. m.p.: 133°C. Yield: 95,3 %. IR (KBr, cm^{-1}): 3033 (C-H), 1730 (C = O), 1582 (C = C), 1291 (C-N), 725 (C-H). ^1H NMR (300 MHz, CDCl_3): δ 7,88 (m, 2H, $J = 9$ Hz, FtH), 7,82 (s, 1H, CH), 7,74 (m, 2H, $J = 8,1$ Hz, FtH), 7,32 (m, 6H, $J = 24,3$ Hz, ArH), 7,15 (m, 6H, $J = 22,5$ Hz, ArH), 7,01 (d, 2H, $J = 8,7$ Hz, ArH), 5,66 (s, 2H, CH_2). ^{13}C -NMR (300 MHz, CDCl_3): δ 166.5, 165.3, 150.2, 146.2, 134.7, 134.4, 131.8, 131.5, 131.2, 129.6, 129.6, 129.0, 126.2, 125.9, 125.1, 125.0, 124.7, 123.8, 120.47, 119.3, 116.1, 43.3 (CH_2). MS m/z (%): 531.38, calculated 531.12.

3-((1,3-dioxoisindolin-2-yl)methyl)-5-((3-phenyl-1H-pyrazol-4-yl)methylene)thiazolidine-2,4-dione (LPSF/FT-09) (**9i**)

$\text{C}_{22}\text{H}_{14}\text{N}_4\text{O}_4\text{S}$. Yellow solid. m.p.: 214°C. Yield: 84,3 %. IR (KBr, cm^{-1}): 3321 (N-H), 3015 (C-H), 1710 (C = O), 1610 (C = C), 1318 (C-N). ^1H NMR (300 MHz, DMSO-d_6): δ 13,82 (s, 1H, NH), 8,24 (s, 1H, CH), 7,87 (m, 4H, $J = 27,9$ Hz, ArH), 7,61 (t, 6H, $J = 39$ Hz, ArH), 5,46 (s, 2H, CH_2). ^{13}C -NMR (300 MHz, DMSO-d_6): δ 166.3, 165.8, 164.3, 134.8, 131.1, 129.0, 128.5, 123.3, 117.1, 112.5, 43.7 (CH_2). MS m/z (%): (M + H)⁺ 431.19, (M + Na)⁺ 453.23, calculated 430.07.

5-((2-chloro-6-methylquinolin-3-yl)methylene)-3-((1,3-dioxoisindolin-2-yl)methyl)thiazolidine-2,4-dione (LPSF/FT-12) (**9j**)

$\text{C}_{23}\text{H}_{14}\text{ClN}_3\text{O}_4\text{S}$. Yellow solid. m.p.: 356°C. Yield: 72,2 %. IR (KBr, cm^{-1}): 1781 (C = O), 1731 (C = O), 1654 (C = C), 1310 (C-N). ^1H NMR (400 MHz, DMSO-d_6): δ 8,10 (s, 1H, ArH), 7,86 (m, 4H, $J = 22,8$ Hz, ArH), 7,56 (s, 1H, ArH), 7,40 (dd, 2H, $J = 10$ Hz, ArH), 7,22 (d, 1H, $J = 8$ Hz, ArH), 5,46 (s, 2H, CH_2), 2,48 (m, 3H, $J = 5,6$ Hz, CH_3). ^{13}C -NMR (300 MHz, CDCl_3): δ 167.6, 166.6, 165.1, 160.6, 142.9, 137.3, 135.1, 134.1, 132.1, 131.3, 129.0, 128.7, 124.6, 123.6, 122.4, 119.2, 115.4, 43.8 (CH_2), 20.4 (CH_3). MS m/z (%): (M + H)⁺ 464.03, calculated 463.03.

3-((1,3-dioxoisindolin-2-yl)methyl)-5-(4-(pyrimidin-5-yl)benzylidene)thiazolidine-2,4-dione (LPSF/FT-15) (**9k**)

$\text{C}_{23}\text{H}_{14}\text{N}_4\text{O}_4\text{S}$. Yellow solid. m.p.: 288°C. Yield: 87,3 %. IR (KBr, cm^{-1}): 3014 (C-H), 1749 (C = O), 1597 (C = C), 1312 (C-N), 727 (C-H). ^1H NMR (300 MHz, CDCl_3): δ 9,25 (s, 1H, ArH), 8,99 (s, 2H, ArH), 7,96 (s, 1H, CH), 7,90 (m, 4H, $J = 8,4$ Hz, ArH), 7,72 (m, 4H, $J = 45,3$ Hz, ArH), 5,69 (s, 2H, CH_2). ^{13}C -NMR (300 MHz, CDCl_3): δ 166.5, 164.8, 158.0, 154.8, 134.5, 133.6, 133.4, 133.0, 131.5, 131.1, 127.6, 123.8, 121.8, 43.5 (CH_2). MS

Declarations

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The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Figures

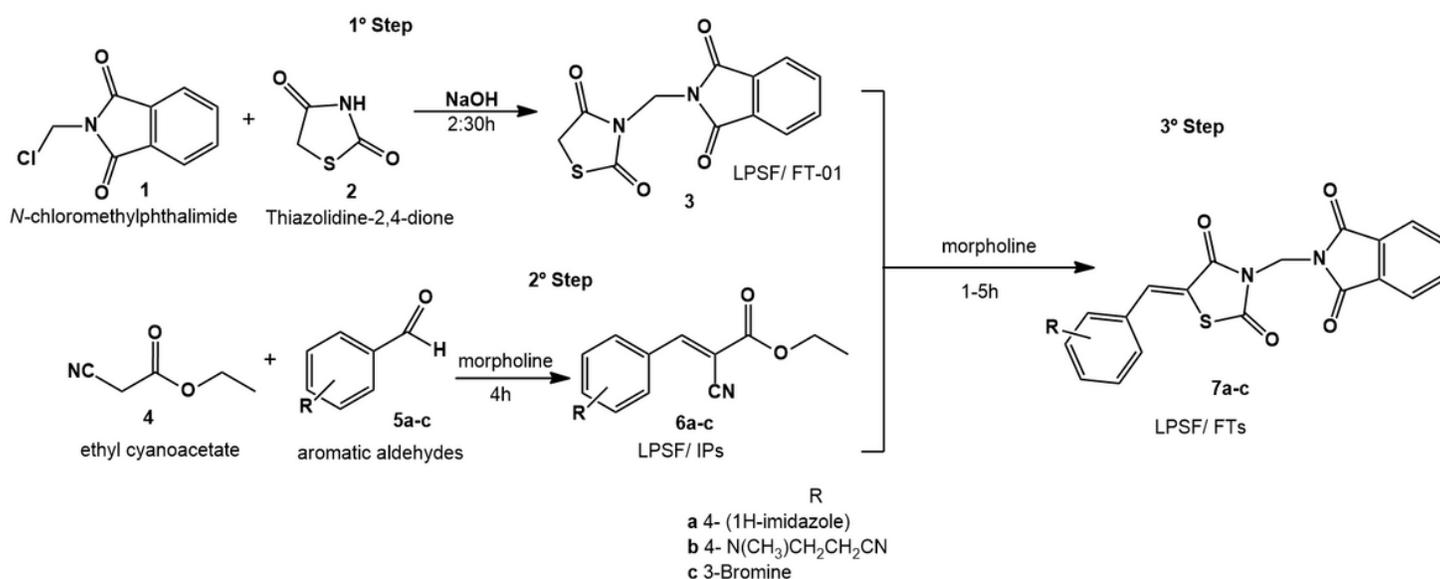


Figure 1

Synthesis of novel phthalimido-thiazolidine derivatives (LPSF/ FTs)

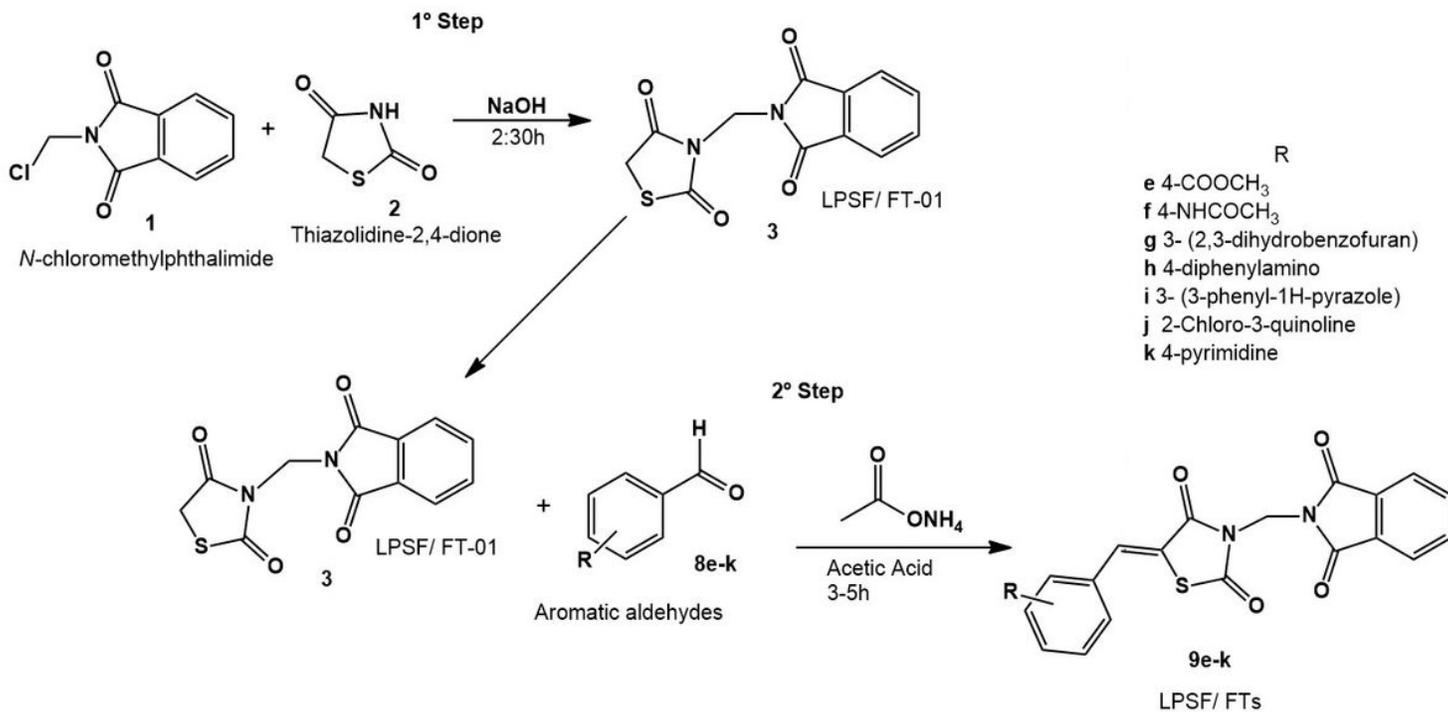


Figure 2

Synthesis of novel phthalimido-thiazolidine derivatives (LPSF/ FTs)

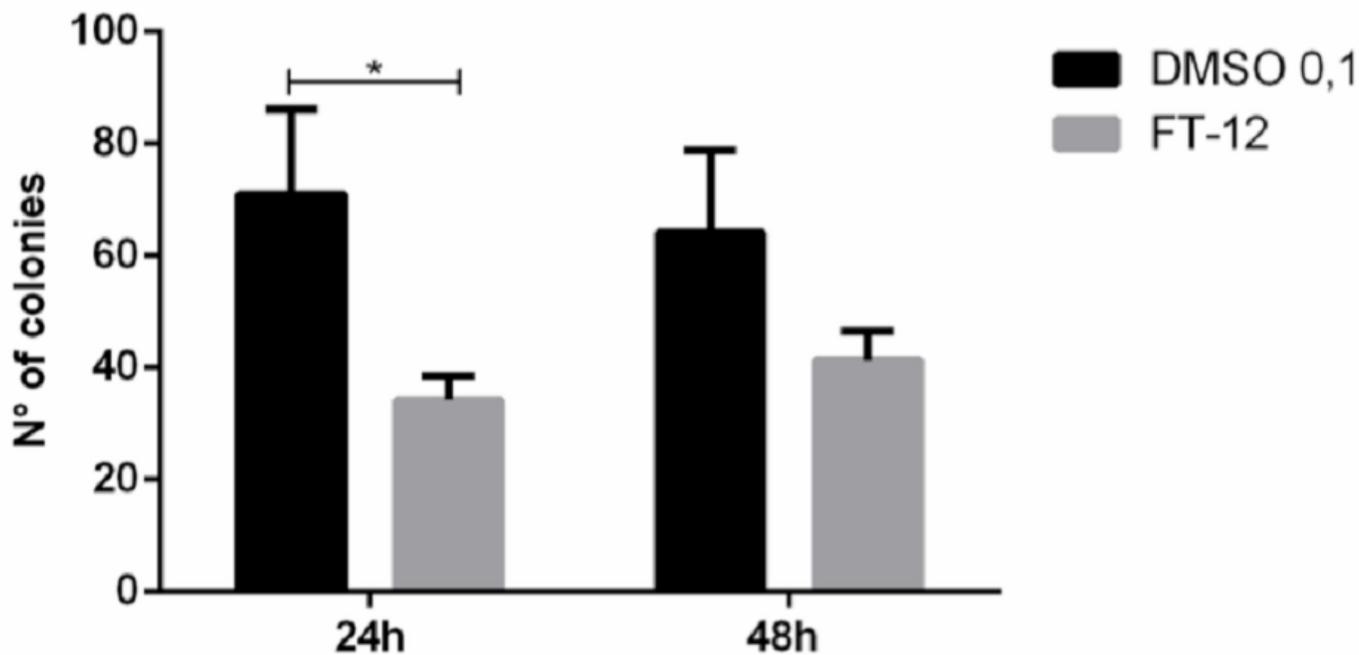


Figure 3

Colony formation assay for analyzing the effect of FT-12 derivative in PC-3 cells.

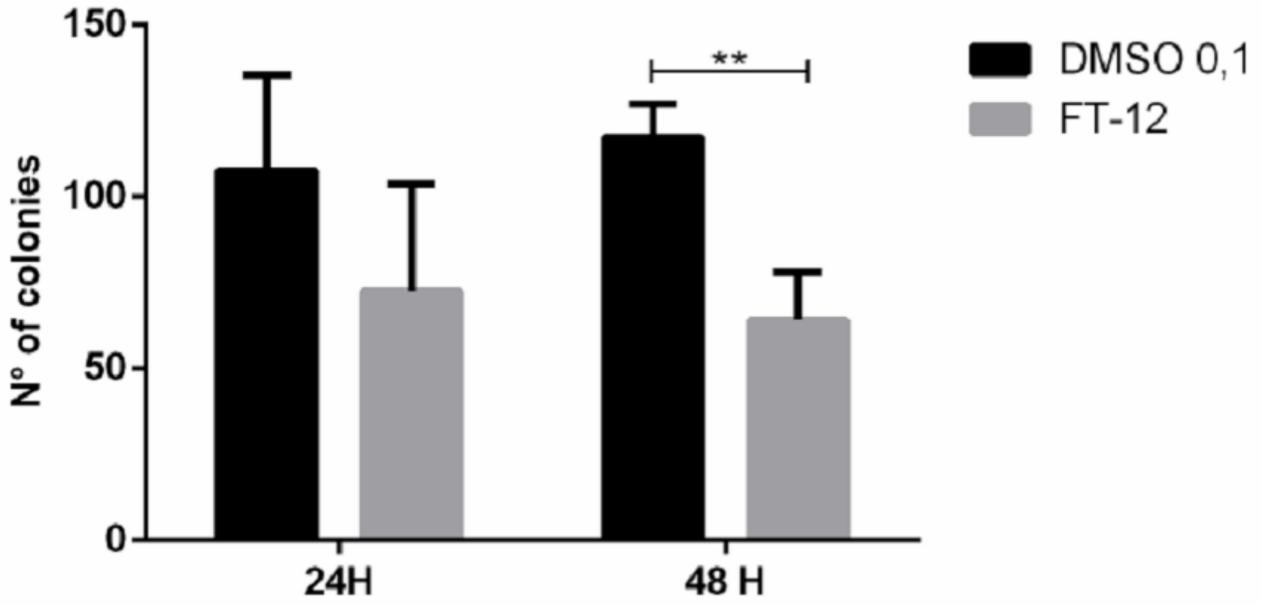


Figure 4

Colony formation assay for analyzing the effect of FT-12 derivative in Panc-1 cells.

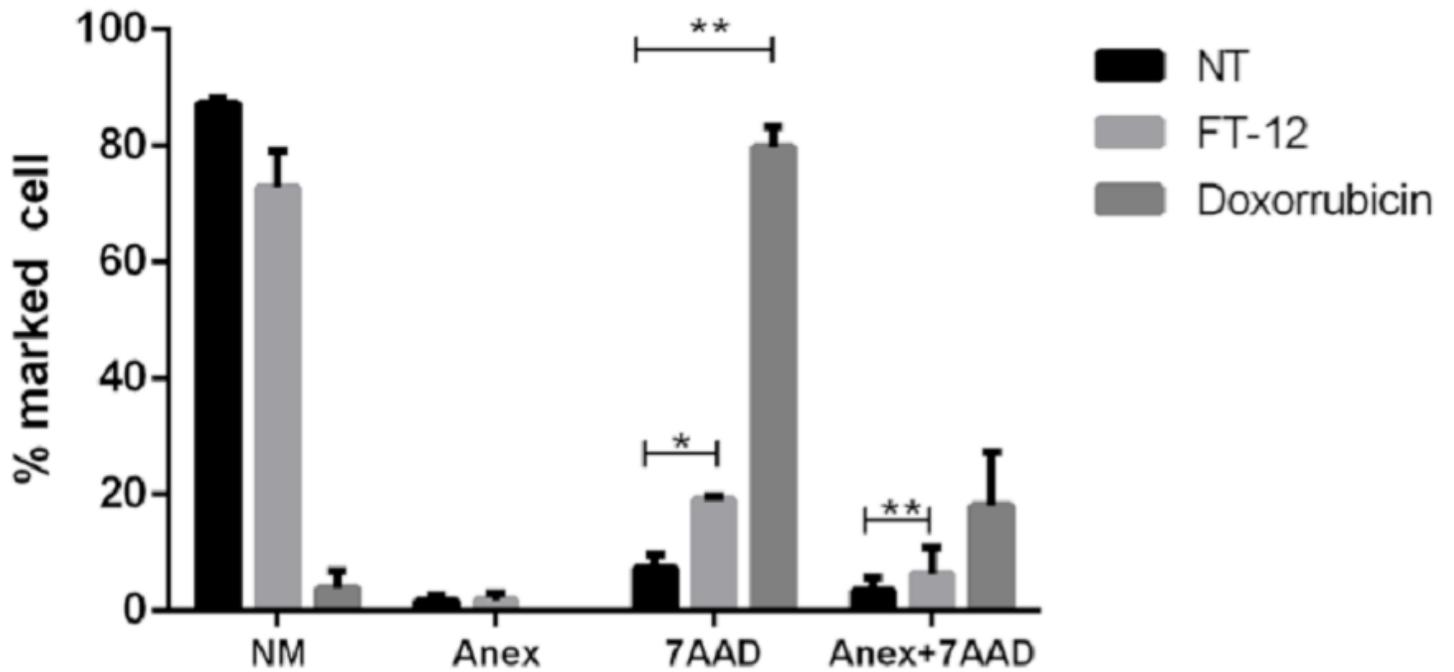


Figure 5

Effect in Panc-1 of FT-12 derivative and doxorubicin in apoptosis/necrosis by flow cytometry.

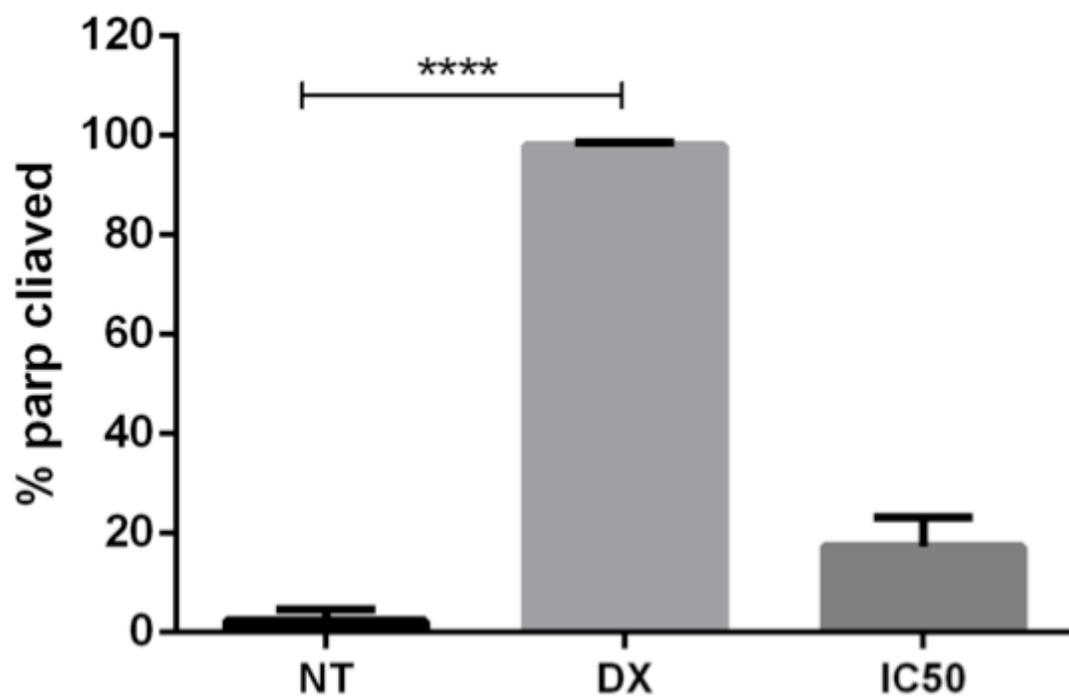


Figure 6

Effect of FT-12 derivative and doxorubicin in Panc-1, PARP cleavage by flow cytometry.

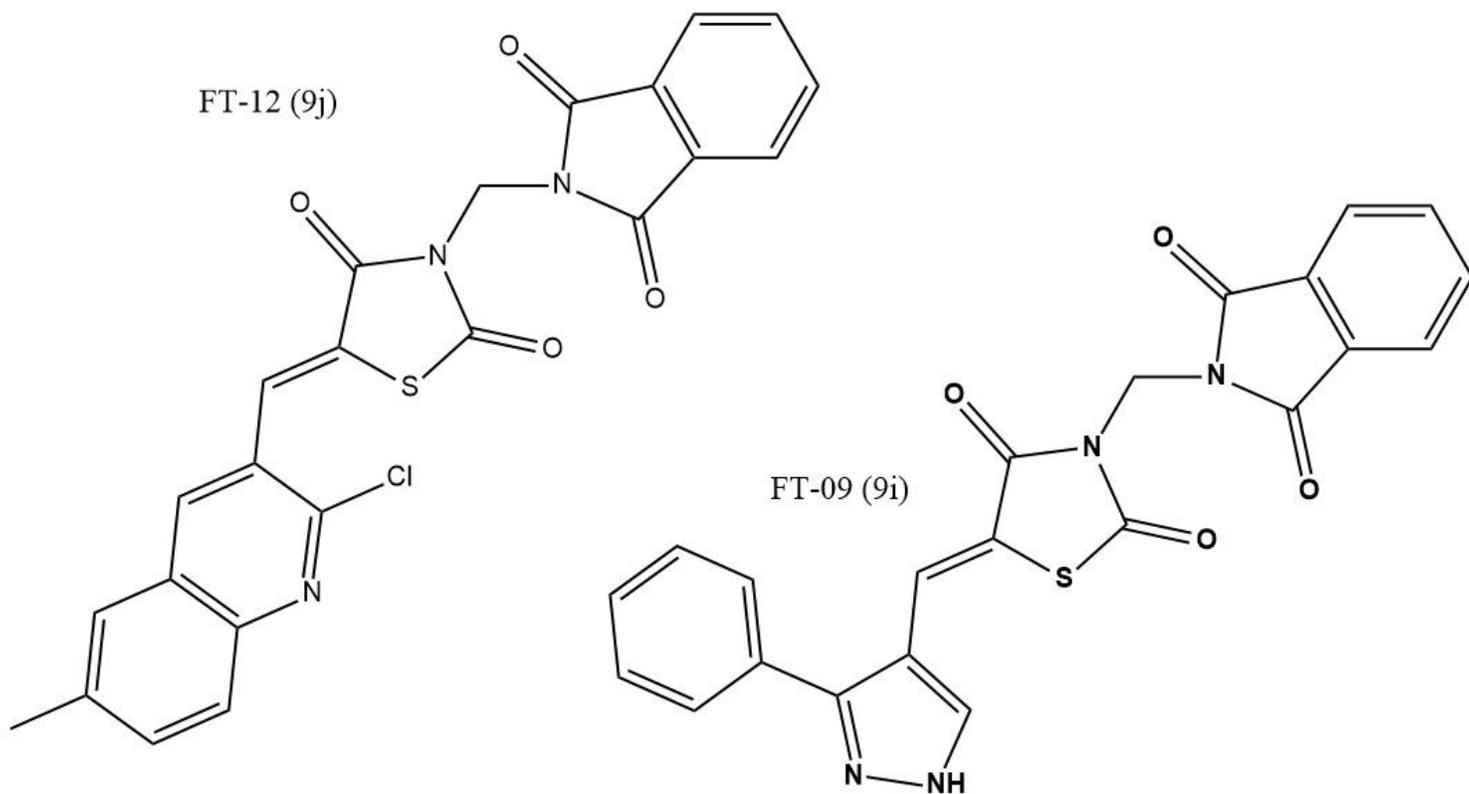


Figure 7

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Chemical structure of FT-09 (9i) and FT-12 (9j) compounds

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