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Research paper

# Discovery of imidazopyridine-pyrazoline-hybrid structure as SHP-1 agonist that suppresses phospho-STAT3 signaling in human breast cancer cells

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ABSTRACT

Signal transducer and activator of transcription 3 (STAT3) promotes breast cancer malignancy and controls key processes including proliferation, differentiation, and survival in breast cancer cells. Although many methods for treating breast cancer have been improved, there is still a need to discover and develop new methods for breast cancer treatment. Therefore, we synthesized a new compound 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)-1-(3-(2,6-dimethylimidazo[1,2-a]pyridin-3-yl)-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (DIP). We aimed to evaluate the anti-cancer effect of DIP in breast cancer cells and clarify its mode of action. We noted that DIP abrogated STAT3 activation and STAT3 upstream kinases janus-activated kinase (JAK) and Src kinases. In addition, DIP promoted the levels of SHP-1 protein and acts as SHP-1 agonist. Further, silencing of SHP-1 gene reversed the DIP-induced attenuation of STAT3 activation and apoptosis. DIP also induced apoptosis through modulating PARP cleavage and oncogenic proteins. Moreover, DIP also significantly enhanced the apoptotic effects of docetaxel through the suppression of STAT3 activation in breast cancer cells.

Overall, our data indicated that DIP may act as a suppressor of STAT3 cascade, and it could be a new therapeutic strategy in breast cancer cells.

## 1. Introduction

Breast cancer is the most prevalent malignancy and commonly diagnosed cancer in women over the world [1,2]. The incidence of breast cancer in women has been gradually increasing by about 0.5% per year since the mid-2000s [3]. Patients are generally treated by using chemotherapy, radiotherapy, and surgical interventions often have severe side effects [4,5]. Although survival rates have increased over the past with the introduction of screening mammography and improved chemotherapy, breast cancer remains the second leading cause of cancer-related death in women [6,7]. There is still need to discover and develop new methods to treat this disease [8–10].

Signal transducer and activator of transcription 3 (STAT3) is a tumorigenic transcription factor that can be aberrantly activated in various tumors such as breast, pancreas, prostate, liver cancer, and leukemia [11,12]. It is known that STAT3 promotes breast cancer malignancy and is overexpressed and constitutively activated in the progression, proliferation, metastasis and resistance of breast cancer [8,13]. STAT3 can be mediated by janus-like kinase (JAK) and non-receptor protein tyrosine kinases Src [14–17]. The activation of STAT3 has

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been demonstrated to contribute to inducing various oncogenic genes and malignant biological behaviors of cancer [18,19]. In previous studies, it has been reported that by various pharmacological agents can induce apoptosis in tumor cells through inhibiting STAT3 activation [11,12,14,20]. In contrast, various tyrosine phosphatases including src-homology region 2 domain-containing phosphatases SHP-1 and SHP-2, and tensin homolog (PTEN), protein tyrosine phosphatase epsilon (PTPε) have been documented to regulating the STAT3 pathway [21–23]. Thus, blockade of STAT3 signaling is an attractive therapeutic approach against human cancers. In this regard, we previously reported a novel structure as inhibitors of p-STAT3 in different cancer cells [24,25]. Further, we also developed novel azaspirane structure that activates phosphatases and targets JAK-STAT3 pathway in hepatocellular carcinoma animal model [26]. In addition, the proof of principle study related to sorafenib and its analogs were reported to show higher SHP-1 activity while inhibiting the phospho-STAT3 signaling in many cancer models [27].

Dovitinib induced the apoptosis by increasing the activity of SHP-1 and decreasing the p-STAT3, which was found to be sensitive towards sorafenib resistant hepatocellular carcinoma cells [28]. Apart from sorafenib, dovitinib and their analogs, zerumbone, a natural electrophlic cyclic keto-compound was found to increase the SHP-1 activity and decreased the p-STAT3 proteins in cancer cells [29]. Recently, we synthesized new compound called 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)-1-(3-(2,6-dimethylimidazo[1,2-a]pyridin-3-yl)-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (DIP) and reported its inhibitory role against p-STAT3 in ER positive breast cancer cells [30]. However, a detailed anti-cancer effect of DIP against human breast cancer cells was unknown. In this study, we evaluated the mode-of-action of DIP compound in detail and found that it activates SHP-1, induced apoptosis, decreased the phosphorylation of JAK2-STAT3 in BC cells (Fig. 1).

## 2. Material and methods

### 2.1. Reagents

Antibodies of JAK2, JAK1, p-JAK2(Tyr1007/1008), p-JAK1 (Tyr1022/1023), p-Src, p-STAT3(Tyr705), and anti-Cyclin D1 were obtained from Cell Signaling Technology (Beverly, MA). Antibodies of STAT3, Src, SHP-1, SHP-2, PTPε, PTEN, PARP, p-p53, Bcl-2, β-actin, Survivin, and COX-2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

### 2.2. Cell culture conditions

Human breast cancer cells (MCF-7, T47D, BT-474, and SK-BR-3) and normal MCF-10A cells were purchased from Korean Cell Line Bank (Seoul, Korea). MCF-7, SK-BR-3, BT-474, cells were cultured in RPMI6140 medium. T47D cells were cultured in DMEM high glucose medium. MCF-10A cells were cultured in DMEM/F-12 medium. All the medium supplemented with fetal bovine serum (FBS, 10%) and penicillin-streptomycin antibiotics (1%). All cells were maintained at 37 °C under 5% $CO_2$ chamber.

### 2.3. MTT assay

MCF-7, T47D, SK-BR-3, and BT-474 cells were treated with (0, 1, 3, 5, 8, 10 μM) of DIP for 24 h. The viability of cells was determined through MTT assay and also half-inhibitory concentration ($IC_{50}$) calculation was performed as described earlier [12].

### 2.4. Western blot analysis

Breast cancer cells were treated as indicated concentrations and time conditions and Western blot analysis for various antibodies was carried out as elaborated earlier [31].

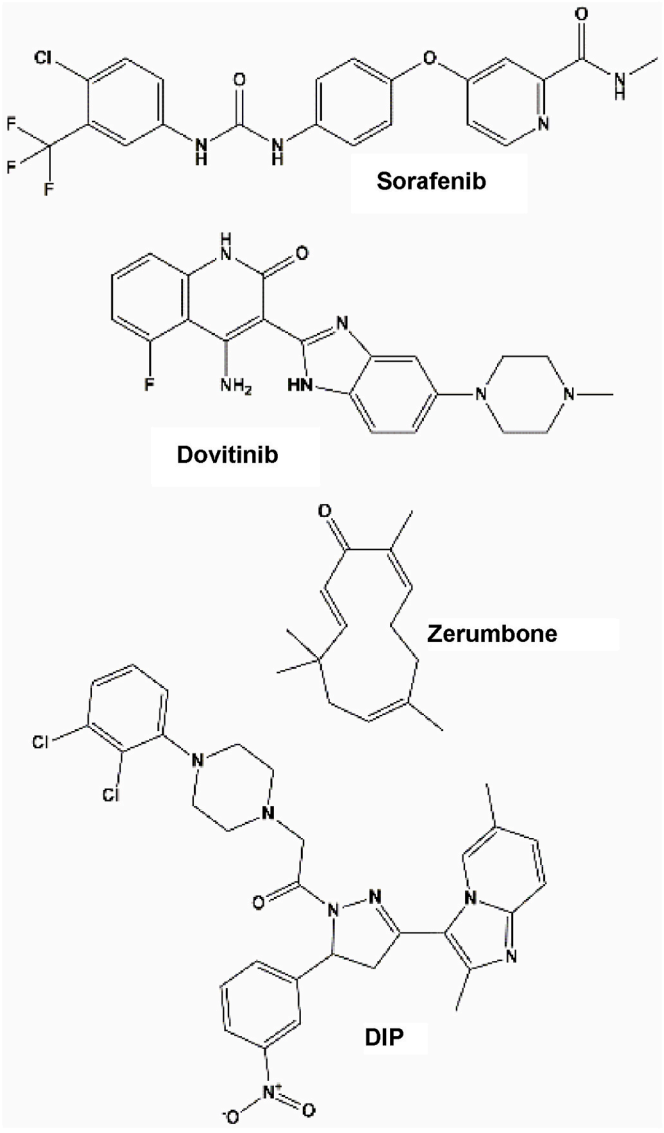


**Fig. 1.** Structures anti-cancer agents reported as SHP-1 agonist.

### 2.5. Immunocytochemistry

Cells were treated with DIP (5 μM) for 6 h. The cells were fixed with 4% paraformaldehyde for 20 min and incubated with Triton X-100 (0.2%) for 10 min. Thereafter, immunocytochemistry as reported previously [12].

### 2.6. Electrophoretic mobility shift assay (EMSA) for STAT3-DNA binding

Human breast cancer cells were treated with DIP (0, 1, 3, 5 μM) for 6 h and STAT3-DNA binding reaction was analyzed through EMSA as described before [11]. Briefly, nuclear extract was prepared and protein-oligonucleotide was loaded on a polyacrylamide gel and transferred to nylon membrane cross-linked by UV (540 nm). STAT3-oligonucleotide probe: 5′-GATCCTTCTGGGAATTCCTAGATC-3′ and 5′-GATCTAGGAATTCCCAGAAGGATC-3′. And Oct-1 was used for loading control (5′-TTCTAGTGATTTGCATTCGACA-3′ and 5′-TGTCGAATGCAAATCACTAGAA-3′).

### 2.7. Cell cycle analysis

The effect of DIP on cell cycle progression was examined by cell cycle analysis as reported before [18].

### 2.8. Annexin V assay

Breast cancer cells were treated with DIP (5 μM) for 24 h and annexin V assay was performed as reported earlier [11].

### 2.9. TUNEL assay

SK-BR-3, T47D, BT-474, and MCF-7 cells were treated with DIP (5 μM) for 24 h. Thereafter, TUNEL assay was performed as described before [12].

### 2.10. Combination therapy with DIP and docetaxel

SK-BR-3 and MCF-7 cells were treated with DIP and docetaxel with various concentrations for 24 h. Cytotoxicity and combination index were evaluated as reported previously [12].

### 2.11. Molecular docking

Molecular docking simulations were performed using established software AutoDock4 tools (v1.5.6) [32] to assess the binding affinity

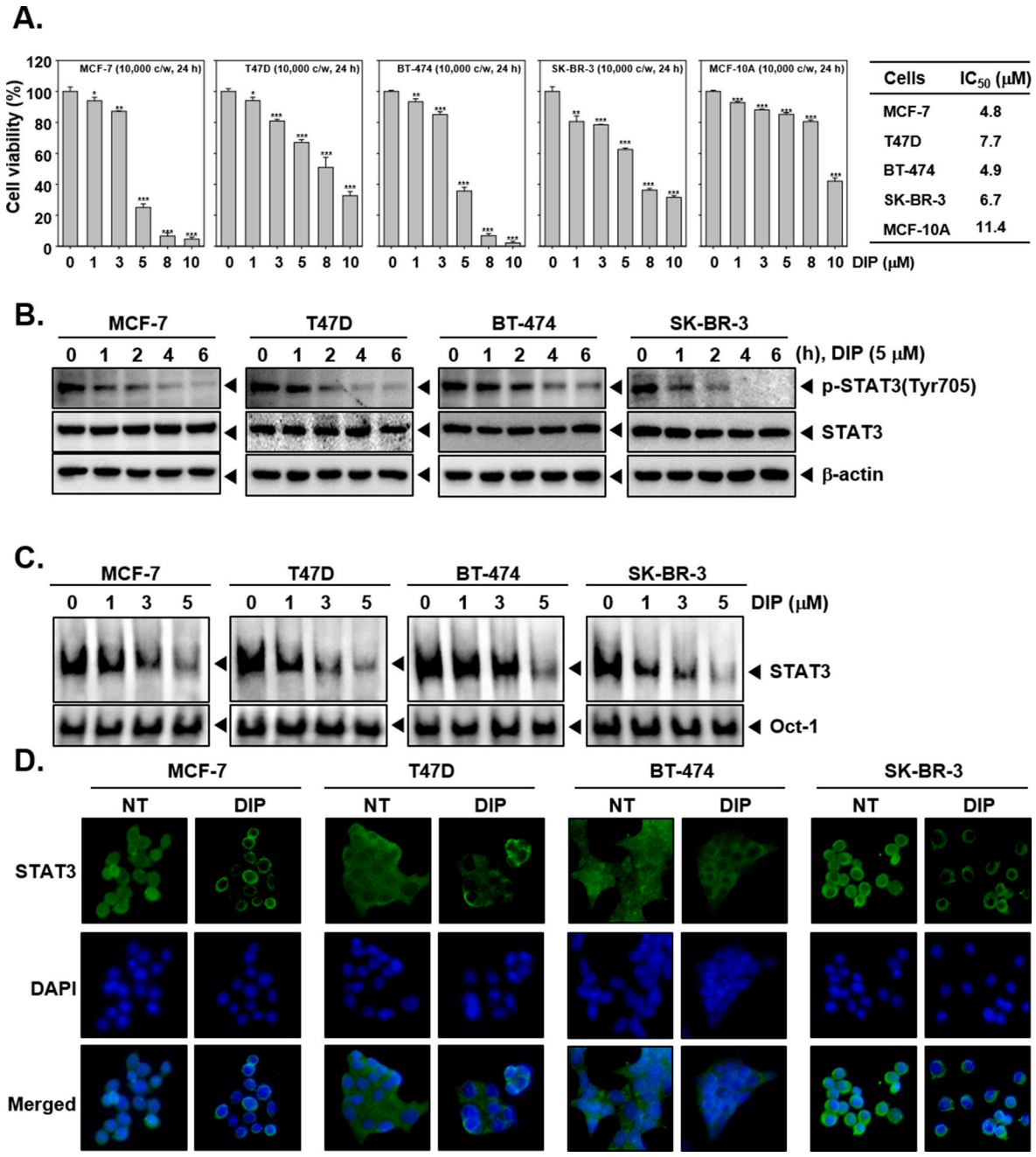


**Fig. 2.** DIP alters STAT3 activation in Human breast cancer cells. **(A)** Human breast cancer cells and normal cells were treated with DIP (0, 1, 3, 5, 8, 10 μM) for 24 h and MTT assay was carried out. Data represents mean ± SD. *$p < 0.05$ vs. non-treated (NT) cells, **$p < 0.01$ vs. NT cells, and ***$p < 0.001$ vs. NT cells. **(B)** Cells were treated with DIP (5 μM) for indicated time conditions and immunoblotting for p-STAT3 and STAT3 was carried out. **(C)** Nuclear extracts were prepared and EMSA was conducted. **(D)** Expression of STAT3 was evaluated by immunocytochemistry.

between the compound DIP and SHP-1. The three-dimensional structure of SHP-1 was obtained from the Protein Data Bank (PDB) (PDB ID: 2B3O) and later water molecules and non-essential components were removed from the protein structure further hydrogen atoms were added to the protein using BIOVIA Discovery Studio. The structure of the compound was generated using chemical drawing software. After the preparations of both compound & protein, Autodock4 software [32] was used for molecular docking simulations. The protein structure was assigned with partial charges. Later the SHP-1 protein structure was set as the recep-tor, while novel compound (DIP) was designated as the ligand. Grid maps were generated around the catalytic domain of SHP-1 to define the docking site and having grid dimension of 70 Å × 60 Å × 90 Å with spacing of 1 Å was set. Lamarckian Genetic Algorithm (LGA) was employed for docking calculations and having 2,500,000 energy evaluations having mutation rate of 0.02 and crossover rate of 0.80 with 10 docking runs were performed to ensure reliable results. Later visualization of docking results was examined using Discovery Studio, Pymol and UCSF Chimera 1.16 [33].

### 2.12. Statistical analysis

The results were expressed as means ± standard deviation (SD), and an analysis of variance (ANOVA) with Bonferroni's test was used for the statistical analysis of multiple comparisons of data. *p*-value of 0.05 or less was considered as significant.

## 3. Results

### 3.1. DIP inhibits the proliferation of human BC cells

Nintedanib, a multikinase inhibitor inhibited TNBC cell proliferation by acting as a SHP-1 agonist [34], and therefore, we tested the compound DIP against human breast cancer cell proliferation by MTT assay. MCF-7, T47D, BT-474, SK-BR-3, or MCF-10A cells were treated with DIP for about 24 h at varied concentration (0–10 μM) and found that it inhibited the proliferation of BC cells with an $IC_{50}$ values in the range of 4–6 μM, indicate that the compound is cytotoxic to BC cells (Fig. 2A).

### 3.2. DIP inhibits the p-STAT3 in human BC cells

We previously reported that the compound DIP inhibited the p-STAT3 in BC cells. Further. We examined the impact of DIP on STAT3 activation in a dose and time dependent studies using Western blotting method, EMSA, and immunocytochemistry. As shown in Fig. 2B, DIP at 5 μM concentration attenuated the phosphorylation of STAT3 either at drug-treatment time increases from 0 to 6 h or drug concentration increase from 0 to 5 μM in MCF-7, T47D, BT-474, and SK-BR-3 cells. The dephsophorylation in BC cells was effective in BC cells in 4 h drug exposure or 5 μM use (Fig. 2B and C). Also, DIP suppressed DNA-binding ability of STAT3 and translocation of STAT3 to the nucleus (Fig. 2D).

### 3.3. DIP inhibits upstream kinases activation

DIP was found to decrease the p-STAT3 in BC cells. However, the mode of action of DIP in BC cells was remained to be unknown. Therefore, we checked weather, DIP could decrease the upstream kinase JAK1 and JAK2 in BC cells as we carried out the experiments reported earlier [35]. MCF-7, T47D, BT-474, SK-BR-3 cells were treated with DIP (0, 1, 3, 5 μM) for 6 h and western blotting was carried out. As shown in Fig. 3A, JAK1, JAK2, Src phosphorylation was down-regulated upon DIP treatment. This indicate that the compound could enter into cells and could dephosphorylate the JAKs and Src genes.

### 3.4. DIP induces SHP-1 protein expression

SHP-1 was well known as prognostic marker and thereby targeting SHP-1 and pSTAT3 signal by DIP in cancer cells could be a valid viable strategy [36]. We therefore analyzed whether STAT3 inhibition caused by DIP involved the protein tyrosine phosphatases (PTPs). MCF-7, T47D, BT-474, and SK-BR-3 cells were treated with sodium pervanadate or DIP. As shown in Fig. 3B, sodium pervanadate alters inhibition of STAT3 activation. The results suggested the possibility of involvement PTPs in DIP-induced inhibition of STAT3 phosphorylation. Next, we examined the impact of DIP on PTPs expression using Western blot analysis. As shown in Fig. 3C, DIP induced SHP-1 expression but did not affect SHP-2, PTPε, PTEN expressions. To confirm whether DIP abrogate STAT3 activation through up-regulating SHP-1 expression, we performed SHP-1 knockdown experiment. As shown in Fig. 3D and E, DIP-induced SHP-1 expression was mitigated in the SHP-1 siRNA transfected cells. But it was not altered in scrambled siRNA transfected cells. Also, DIP-induced p-STAT3 inhibition was attenuated in SHP-1 siRNA transfected cells. These results suggested that DIP modulated STAT3 phosphorylation through up-regulating SHP-1 expression.

### 3.5. In silico analysis of a novel compound DIP targeting the SHP-1

Src homology region 2 domain-containing protein tyrosine phosphatase-1 (SHP-1) is a critical protein involved in the regulation of various cellular processes, including cell growth, immune response, and apoptosis. Modulation of SHP-1 activity through agonists has the potential to impact these processes, making it a promising target for drug development [37]. In this study, we investigated the binding affinity of a novel compound (DIP) to SHP-1 to determine its potential as an agonist by comparing it with the known agonist called SC-43 [38].

The binding energy for the interaction between the DIP and SHP-1 was determined by molecular docking simulations to be −7.09 kcal/mol, while the binding energy for the agonist SC-43 was found to be −5.52 kcal/mol, which is lower than that of the DIP molecule (Fig. 4A). This indicating that the compound DIP has a strong affinity for SHP-1. The key interactions between the compound and SHP-1 also showed the formation of a hydrogen bond between the nitro group and ARG-21 having a bond distance of 2.28 Å, formation of the attractive charge and the π -anion bond between the N of the yrazoline with ASP-185, as well as the dichlorophenyl group of the piperazine, which is having the bond distances of 3.89 Å and 3.73 Å respectively.Further hydrophobic interaction were observed with HIS-6,ARG-7,LEU-100,LYS-102,PRO-105 and LEU-187 (Fig. 4B and C). In this study, we assessed the binding affinity of DIP to SHP-1 and compared it to the known SHP-1 agonist, SC-43. Critical interaction analysis highlighted specific residues involved in the binding process. Finally this finding suggests that the novel compound DIP has the potential to act as an agonist for SHP-1.

### 3.6. DIP promotes apoptotic cell death

Apoptosis is a programmed physiological cell suicide process to maintain homeostasis and eliminate cells that contain potentially dangerous mutations [39,40]. Previous studies confirmed that STAT3 activation induces dysregulation of cell cycle control and apoptosis, representing an important molecular event that promotes cell growth and survival and contribute to tumorigenesis [11,41–43]. Therefore, we determined the impact of DIP on apoptotic cell death. Fort this purpose, the MCF-7, T47D, BT-474, and SK-BR-3 cells were treated with DIP (0, 1, 3, 5 μM) for 24 h and analyzed for cell cycle distribution, annexin V and TUNEL assays. As shown in Fig. 5A, DIP induced accumulation of subG1 phase in all cell lines and also induced G0/G1 phase in MCF-7 and T47D cells. DIP also induced late apoptosis in MCF-7, T47D, BT-474 cells and induced early and late apoptosis in SK-BR-3 cells (Fig. 5B). The results of TUNEL assay also suggested that DIP treatment increased apoptotic cell death (Fig. 5C).

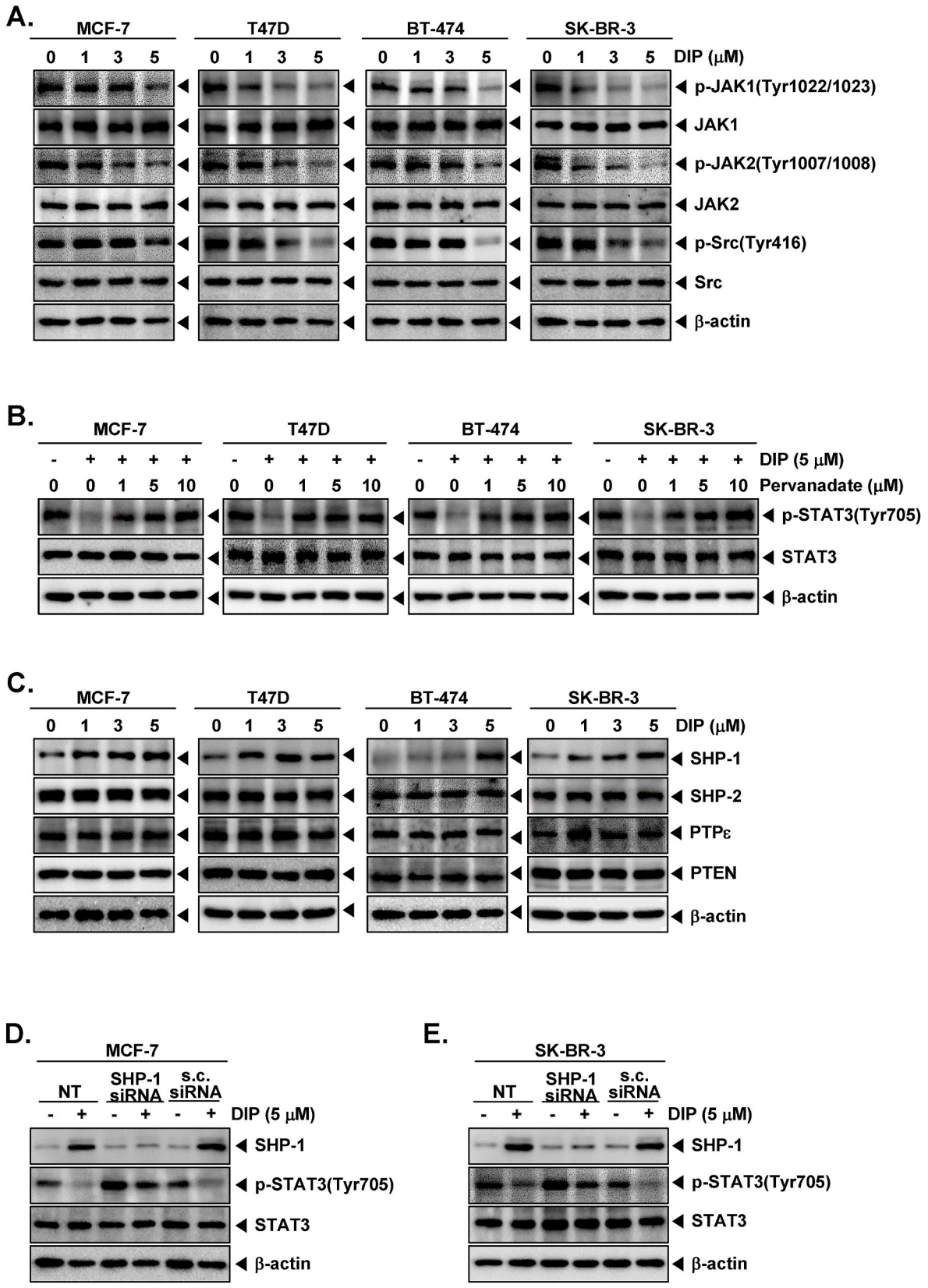


**Fig. 3.** DIP increases the level of SHP-1. **(A)** MCF-7, T47D, BT-474, SK-BR-3 cells were treated with DIP (0, 1, 3, 5 μM) for 6 h and immunoblotting was performed. **(B)** Cells were treated with DIP and pervanadate as indicated concentrations for 6 h and Western blotting for p-STAT3(Tyr705) and STAT3 was performed. **(C)** Cells were treated with DIP for 6 h and immunoblotting was executed. **(D and E)** MCF-7 and SK-BR-3 cells were transfected with SHP-1 siRNA or scrambled siRNA (100 nM). After 24 h, the cells were treated with DIP (5 μM) for 6 h and Western blotting was carried out.

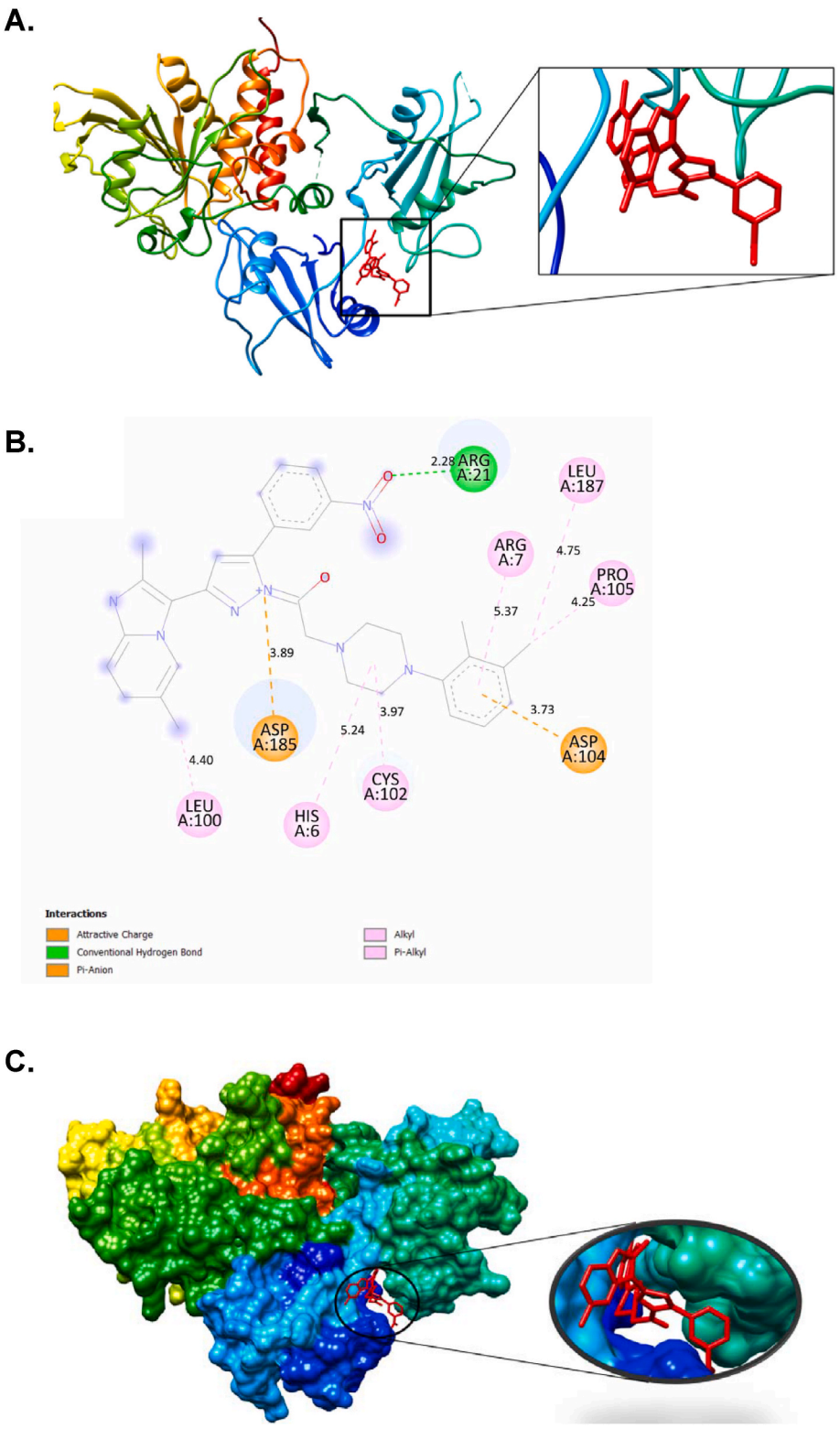


**Fig. 4.** **(A)** Cartoon representation of the docked compound DIP (red) with SHP-1. **(B)** Showing critical interactions between the compound and the residues of SHP-1. **(C)** 3D surface view of the docked compound with SHP-1 and its enlarged view for better visualization.

### *3.7. DIP modulates the apoptotic markers*

Apoptosis is primarily characterized by the enzymatic cleavage of poly (ADP-ribose) polymerase (PARP) by caspase-3 [44]. During apoptosis, cleavage of PARP in fragments of 89 and 24 kDa has enables repair DNA damage and stability [45]. Therefore, PARP and caspase-3 has become a hallmark in apoptosis evaluation. In cancer, this process is greatly impaired and evasion, resulting in increased survival of cancer cells by modulating pro-apoptotic and anti-apoptotic proteins [36–38]. As shown in Fig. 6A and B, DIP induced PARP cleavage and p53 expression. DIP also down-regulated anti-apoptotic proteins expression (Fig. 6C). Additionally, SHP-1 knockdown attenuated the induction of

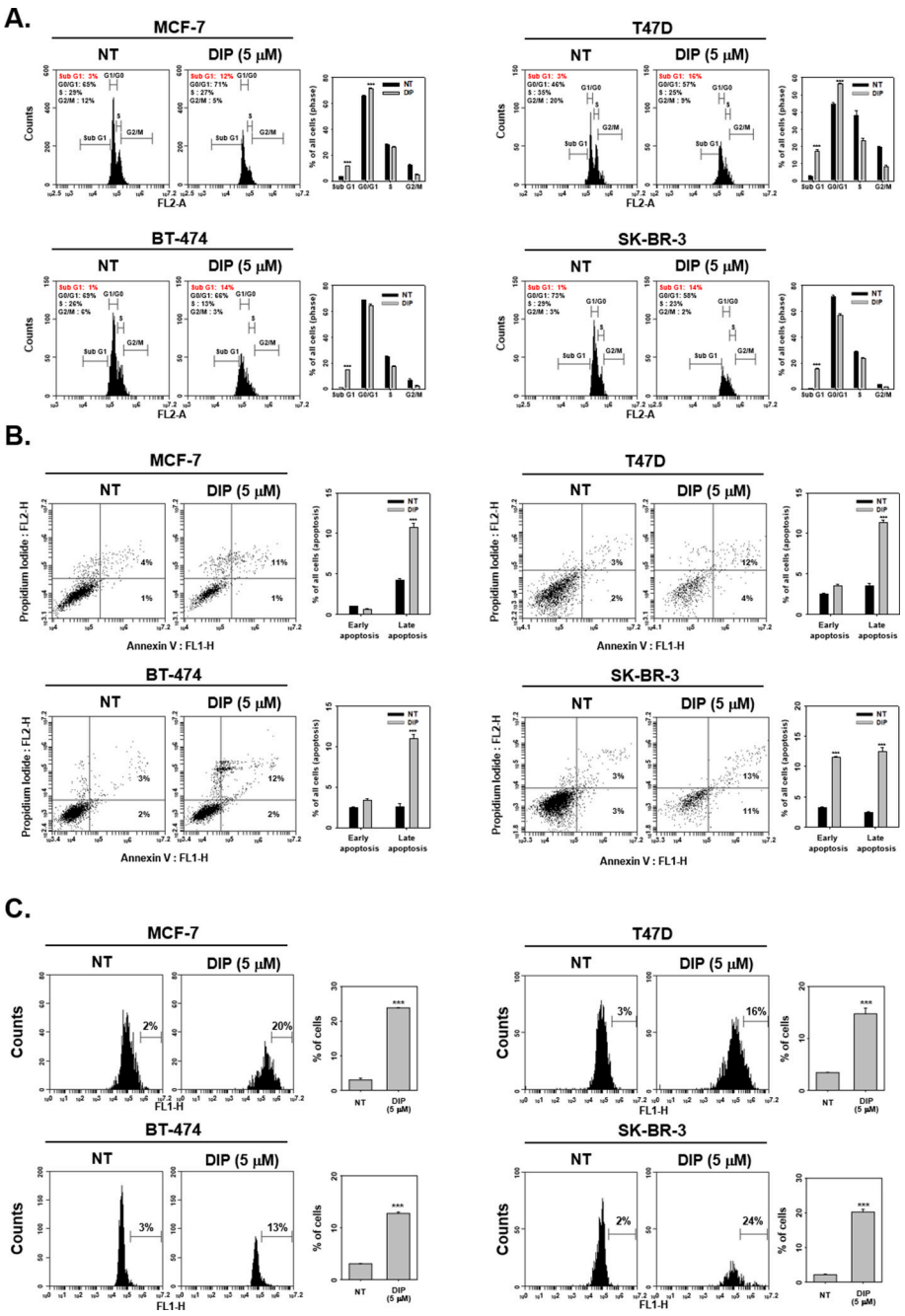


**Fig. 5.** DIP induces apoptosis in breast cancer cells. All cells were treated with DIP (5 μM) for 24 h. **(A)** Cells were incubated with RNase A and cell cycle analysis was performed by Flow cytometer. **(B)** The cells were stained with PI and FITC for 15 min and annexin V assay was carried oute. **(C)** Cells were stained with TUNEL assay reagent. Then TUNEL assay was conducted by Flow Cytometer.

PARP cleavage by DIP (Fig. 6D and E).

### 3.8. DIP enhances anti-cancer effects of docetaxel

The synergistic effect of SHP-1 agonist and docetaxel was found to modulate the STAT3 signaling and induced the apoptosis in BC cells [41]. Therefore, we examined the synergism of DIP and docetaxel induction of apoptosis in BC cells. For this purpose, MCF-7 and SK-BR-3 cells were treated with various combination of DIP and docetaxel for 24 h and synergistic cytotoxicity was evaluated. As shown in Fig. 7A and B, the optimal ratio of combination was found to be DIP (2 μM) and docetaxel (1 nM). Thereafter we demonstrated that DIP and docetaxel combination treatment could significantly induce apoptosis by cell cycle analysis and Western blot analysis. As shown in Fig. 7C and D, subG1 phase arrest was more substantial in the DIP and docetaxel combination group compared with single treatment. As shown in Fig. 7E–J, DIP and docetaxel combination treatment attenuated STAT3 activation greater extent as compared to single treatment. Also, combination treatment promoted PARP cleavage and reduced the expression of oncogenic apoptotic proteins.

## 4. Discussion

Although many methods for treating breast cancer have been improved, there is still a need to discover and develop new methods for treating this disease [8–10]. In a previous study, we synthesized a new

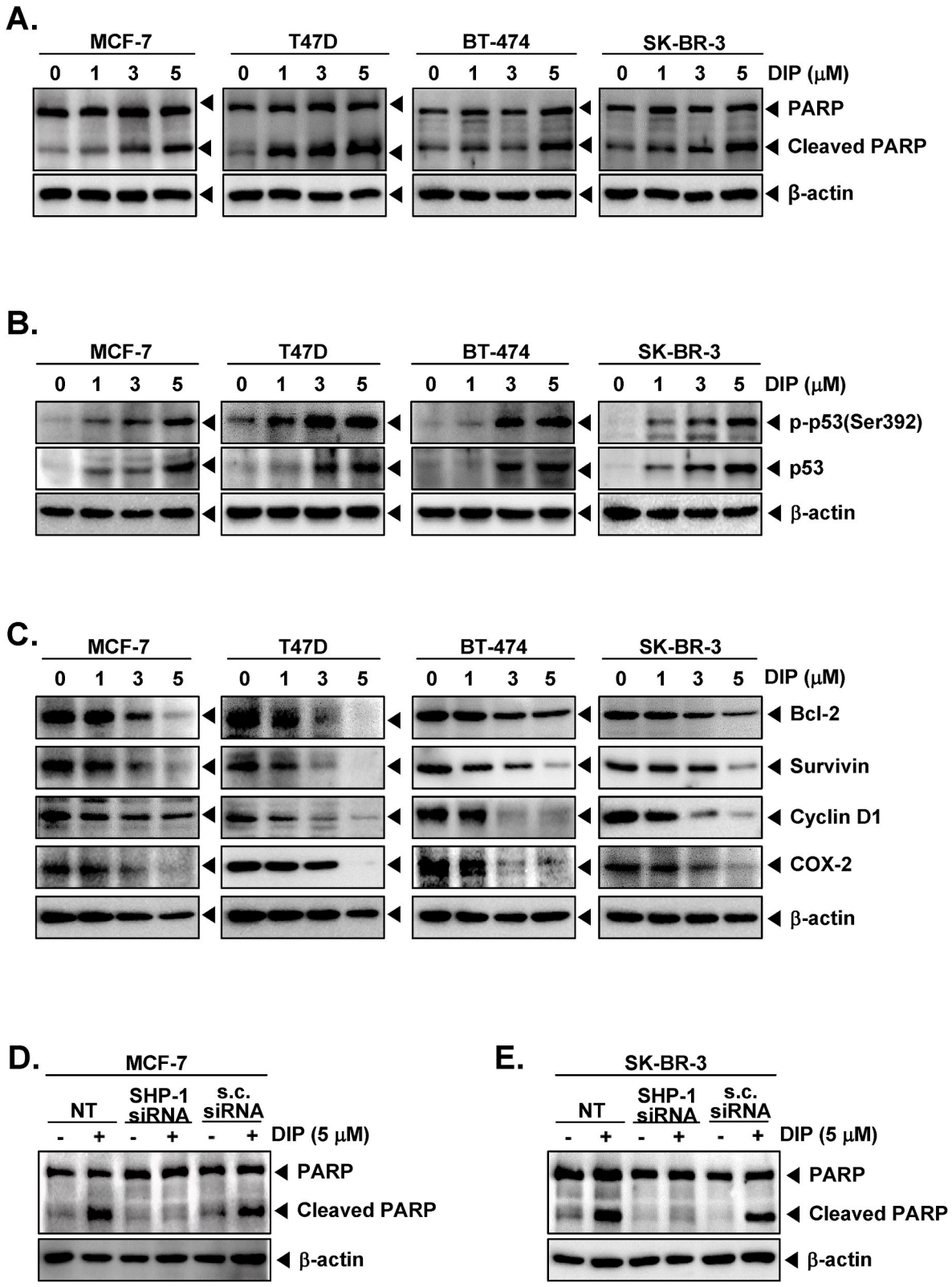


**Fig. 6.** DIP decreases the expression of oncogenic proteins. (A–C) Human breast cancer cells were treated with DIP (0, 1, 3, 5 μM) for 24 h. Immunoblotting for PARP, p-p53, p53, COX-2, Bcl-2, Cyclin D1, and Survivin was carried out. **(D and E)** MCF-7 and SK-BR-3 cells were transfected with SHP-1 siRNA or scrambled siRNA (100 nM) for 24 h. After that, cells were treated with DIP (5 μM) for 24 h and Western blot analysis was conducted.

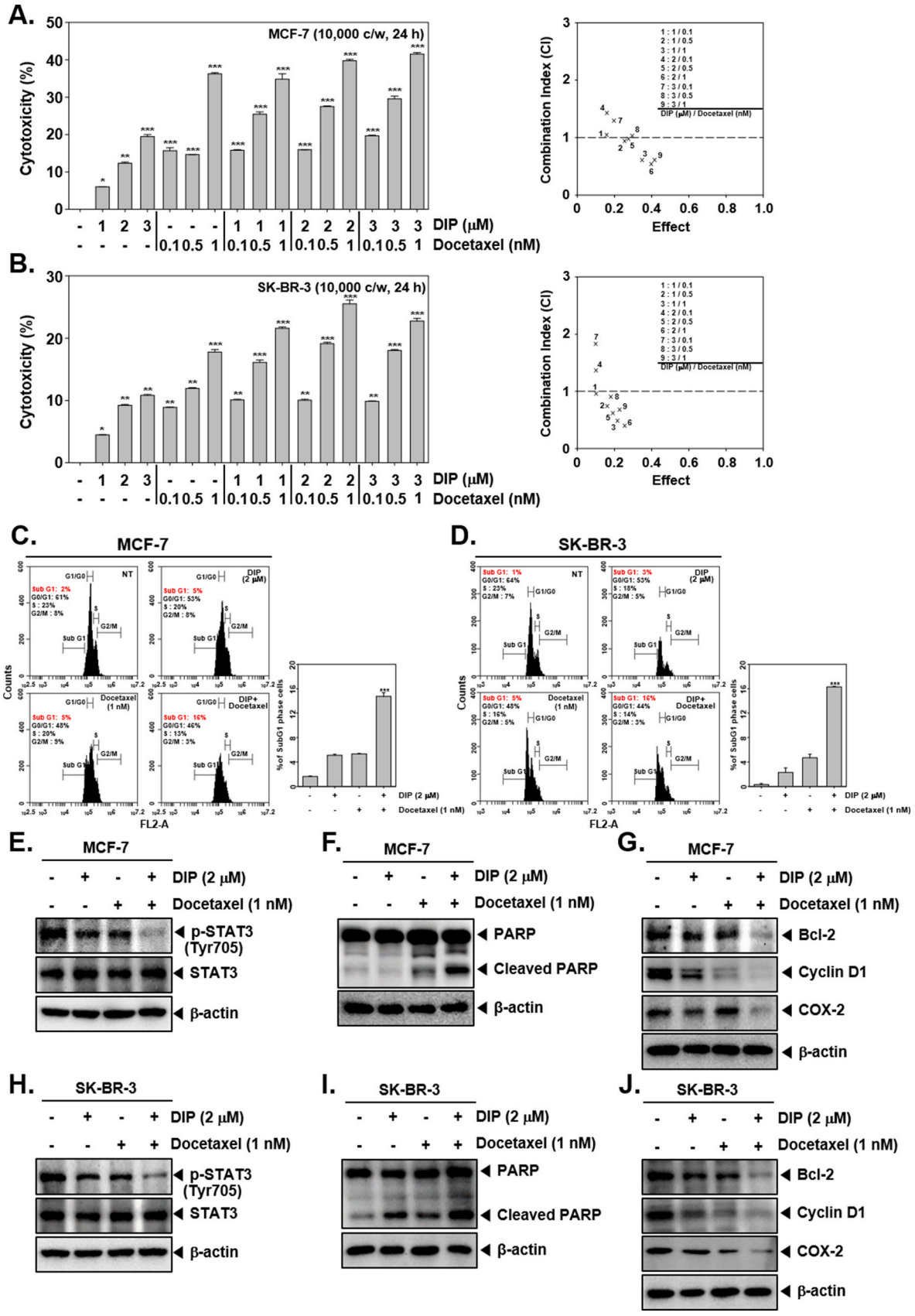


**Fig. 7.** DIP potentiates the anti-cancer effects of docetaxel in MCF-7 and SK-BR-3 cells. (A and B) MCF-7 and SK-BR-3 cells were treated with DIP and docetaxel at the indicated concentrations for 24 h and MTT assay was conducted. The average of CI values about various combination show that the optimal combination ratio was 2 μM of DIP and 1 nM of docetaxel. **(C and D)** The Cells were treated with DIP (2 μM) and docetaxel (1 nM) for 24 h and cell cycle analysis was performed. **(E–J)** Cells were treated with DIP (2 μM) and docetaxel (1 nM) for 6 or 24 h and Western blot analysis for various proteins was conducted.

compound, DIP, which inhibits STAT3 phosphorylation in ER-positive breast cancer cells. However, studies on the anti-cancer activity of DIP and its STAT3 inhibitory effect on other types of breast cancer cells have not been performed. The purpose of this study was to elucidate the anti-cancer effect of DIP in several breast cancer cells and clarify its mode of action.

Overexpressed and constitutively activated STAT3 is involved in breast cancer progression, proliferation, metastasis and chemoresistance [8,13]. A number of previous studies have found that pharmacological agents can modulate the activation of STAT family members and attenuate tumorigenesis [11,12,14]. Jung et al., reported that fangchinoline promoted apoptosis and inhibited cell proliferation through diminishing STAT3 phosphorylation in multiple myeloma [11]. Ginkgolide C exhibit anti-neoplastic actions via abrogation of STAT3 signaling cascade in non-small cell lung cancer model [46]. We found that DIP could suppress STAT3 phosphorylation in human breast cancer MCF-7, T47D, SK-BR-3, and BT-474 cells. Additionally, we noted that DIP attenuated the DNA-binding properties and nuclear localization of STAT3. Next, we examined how DIP inhibits the STAT3 activation mechanically. The JAK and Src activation can promote STAT3 phosphorylation in diverse tumor cell lines, including breast cancer cells [16,17,47,48]. We found that DIP could substantially abrogate JAK1, JAK2, and Src phosphorylation in breast cancer cells.

Various PTPs have been reported that can regulate STAT3 signaling [22]. We also demonstrated that DIP-induced attenuation of STAT3 activation may be caused by modulating PTPs, especially SHP-1. SHP-1 has been found to negatively regulate STAT3 activation [22,37]. It has been suggested that various pharmacological agents may exert their anti-neoplastic effects via inducing expression [31,49,50]. Mohan et al., reported that crocetin imparted anti-proliferative effect through up-regulation of SHP-1 [51]. Interestingly, DIP could substantially promote the expression of SHP-1 proteins, but silencing of this phosphatase could alter the DIP-mediated abrogation of STAT3 activation and cellular apoptosis. These findings suggest that DIP attenuated STAT3 activation by promoting the SHP-1 protein that mediates apoptosis.

Since apoptosis is one of the important systemic anti-tumor processes, the apoptotic effect by DIP was also studied in detail. We found that DIP decreased the expressions of Bcl-2, Survivin, Cyclin D1, and COX-2. In parallel, the increase expression of pro-apoptotic proteins such as Bax and cleavage of PARP were observed. These observations confirm with apoptosis induction as indicated by the increase in the number of apoptotic cells as detected by cell cycle, annexin V and TUNEL assays. These results suggested that DIP induces apoptotic activity by modulating the apoptosis-related proteins. Docetaxel is a classic chemotherapy drug used for the treating cancers such as breast cancer, prostate cancer, non-small cell lung cancer [52]. We further demonstrated that DIP enhanced the apoptotic and cytotoxic actions of docetaxel by affecting STAT3 signaling pathway in breast cancer cells. The combination of DIP (2 μM) and docetaxel (1 nM) could suppress the activation of STAT3. Moreover, co-treatment up-regulated apoptosis through the suppression of diverse oncogenic proteins.

Overall, our findings indicate that DIP can serve as an efficient blocker of STAT3 through inducing expression of SHP-1 protein in breast cancer cells. We found that DIP treatment induces inactivation of STAT3 by inducing SHP-1 in breast cancer cells, thereby mediating apoptosis. It can also significantly enhance the apoptotic effects of docetaxel. Through further studies are required in preclinical settings, our results support the possibility that novel therapeutic option against breast cancer. In this report, we discovered the compound named DIP as SHP-1 agonist. Since SHP-1 agonists were rarely found in drug development procedures, we herein, reporting that DIP enhanced SHAP-1, modulated JAK or Src kinase-STAT3 pathways in BC cells. Our bioinformatic analysis found that the compound DIP could bind near the active site of DIP, and silencing of SHP-1 opposed the DIP-induced p-STAT3, and apoptosis indicated that the compound specificity towards SHP-1. DIP was found to induce apoptosis, cleaved PARP, and modulated other oncogene markers. Moreover, DIP and docetaxel showed synergism in induction of apoptosis and suppression of STAT3 in BC cells. Overall, we found a new SHP-1 agonist called DIP, which could be used to develop a novel structure that served in development of SHP-1 and JAK-STAT3 pathway modulators to be used in BC cell chemotherapy.

## CRediT authorship contribution statement

Conceptualization, methodology, original draft preparation, and writing—review and editing: M.H.Y., G.S., and K.S.A.; Formal analysis and investigation: M.H.Y., S.B., A.R., A.K.M, V.P., B.B., and P.E.L.; Funding acquisition, resources, and supervision: K.S.A. All authors have read and agreed to the published version of the manuscript.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbi.2023.110780.

## Abbreviations used

| | |
|---|---|
| STAT3 | Signal transducer and activator of transcription 3 |
| JAK | Janus kinase |
| PTP | Protein tyrosine phosphatase |
| SDS-PAGE | sulfate-polyacrylamide gel electrophoresis |
| EMSA | electrophoretic mobility shift assay |
| c/w | Cells per well |
| DAPI | 4′,6-Diamidino-2-Phenylindole, Dihydrochloride; |
| FBS | Fetal bovine serum |
| PARP | Poly (ADP-ribose) polymerase |
| NT | non-treated |

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