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# Investigation of the potential antitumor activity of PLK1 inhibitor SBE13 in colon cancer cell line HT29

**Muhammed Gömeç[1a*], Fatih Yulak[2b], Mustafa Ergül[3c]**

*[1] Department of General Surgery, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Turkey, [2] Department of Physiology, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Turkey, [3] Department of Biochemistry, Faculty of Pharmacy, Sivas Cumhuriyet University, Sivas, Turkey*

**Corresponding author*

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## ABSTRACT

***Background:*** High levels of Polo-like kinase 1 (PLK1), which are abnormally expressed in many tumor types, are known to contribute to tumorigenesis and poor prognosis. Therefore, specific targeting of PLK1 is an important strategy for cancer therapy. This study, it was aimed to investigate the cytotoxic effect of SBE13, one of the PLK1 inhibitors, against HT29 colon adenocarcinoma cells and its apoptotic potential.
***Methods:*** The cytotoxic effect of SBE13 on HT29 was determined by XTT colorimetric assay. Flow cytometry was also used to determine apoptosis.
***Results:*** SBE13 showed a dose-dependent cytotoxic effect in HT29 cells and its IC50 value was calculated as 11.79 µM for 48 h. Moreover, the Annexin V binding assay revealed that SBE13 treatment significantly increased apoptosis in HT29 cells.
***Conclusion:*** Generally, SBE13 exerts a cytotoxic effect promoted by apoptosis in colon cancer cells HT29. Although the anticancer efficacy of SBE13 in colon cancer is promising, this potential effect should be reinforced by further studies.

**Keywords**: Apoptosis, Colon Cancer, PLK1 inhibition, SBE13.

# Kolon Kanseri Hücre Hattı HT29'da PLK1 İnhibitörü SBE13'ün Potansiyel Antitümör Aktivitesinin Araştırılması

*Süreç*

*Geliş: 21/04/2022*
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## ÖZ

***Amaç:*** Birçok tümör tipinde anormal şekilde eksprese edildiği bulunan Polo benzeri kinaz 1'in (PLK1) yüksek seviyelerinin tümör oluşumuna ve kötü prognoza katkıda bulunduğu bilinmektedir. Bu nedenle, PLK1'in spesifik olarak hedeflenmesi, kanser tedavisi için önemli bir stratejidir. Bu çalışmada, PLK1 inhibitörlerinden SBE13'ün HT29 kolon adenokarsinom hücrelerine karşı sitotoksik etkisinin ve apoptotik potansiyelinin araştırılması amaçlanmıştır.
***Yöntemler:*** SBE13'ün HT29 üzerindeki sitotoksik etkisi, XTT kolorimetrik yöntem ile belirlendi. Apoptozu belirlemek için akış sitometrisi kullanıldı.
***Bulgular:*** SBE13, HT29 hücrelerinde doza bağlı sitotoksik etki gösterdi ve $IC_{50}$ değeri 48 saat süreyle 11.79 µM olarak hesaplandı. Ayrıca Annexin V bağlanma tahlili, SBE13 tedavisinin HT29 hücrelerinde apoptozu önemli ölçüde arttırdığını ortaya koydu.
***Sonuç:*** Genel olarak SBE13, HT29 kolon kanseri hücrelerinde apoptoz tarafından teşvik edilen sitotoksik bir etki gösterir. SBE13'ün kolon kanserinde antikanser etkinliği umut verici olsa da, bu potansiyel etki daha ileri çalışmalarla güçlendirilmelidir.



***Anahtar sözcükler:*** Apoptoz, kolon kanseri, PLK1 inhibisyonu, SBE13.

[a] *muhammedgomec@cumhuriyet.edu.tr* *https://orcid.org/0000-0002-9127-3201* [b] *fatihyulak@gmail.com* *https://orcid.org/0000-0003-3708-6752*
[c] *m.ergul@yahoo.com.tr* *https://orcid.org/0000-0003-4303-2996*

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## Introduction

Cancer is a public health problem that causes serious economic and social problems. It causes serious mortality and morbidity and its prevalence continues to increase day by day. In addition to surgery, non-surgical treatment approaches such as chemotherapy, immunotherapy and radiotherapy have been developed in cancer treatment [1]. Of many cancer types, colon cancer is the third most frequently diagnosed and remains the second leading cause of cancer-related death [2].

Although many chemotherapeutic agents are used in the treatment of colon cancer, the search for the ideal chemotherapeutic agent continues. In this context, many pathways and mechanisms come to the fore. One of these therapeutic approaches is polo-like kinase 1 (PLK1). Polo-like kinases (PLKs) consist of five families of serine/threonine kinases (PLKs1-5) with different functions and expression patterns in mammalian cells [3]. PLKs are critical in detecting mitosis and DNA damage. One of the PLKs, PLK1, is the most studied and best characterized in the pathogenesis of cancer.

It is known that the loss of apoptosis capacity in cells contributes significantly to the development and proliferation of cancer cells [4]. It has been shown that PLK1 is overexpressed in many cancer types, especially colorectal, breast, ovarian, endometrial, and stomach cancers [5,6]. Therefore, induction of apoptosis has become one of the main strategies in the development of new anti-cancer agents [7]. Studies have shown an increase in apoptosis in combination treatments with PLK1-based drugs [3]. In some studies, the anticancer activities of some PLK1 inhibitors such as BI2536, BI6727 (Volasertib), ON01910.Na, HMN-214, NMS-P937, and TKM-080301 were investigated. [8,9]. The results found showed that PLK1 inhibitors could be promising in the treatment of cancer. Studies have shown that a decrease in PLK1 causes a decrease in cell viability and apoptosis. The obtained results show that PLK1 inhibitors are promising in cancer treatment [9]. One of the PLK1-specific inhibitors is SBE13 [10]. In some studies, it has been shown to induce anticancer activity and apoptosis in various tumor cells.

This study, it was aimed to investigate the possible cytotoxic effect of SBE13, a PLK1 inhibitor, on the colon cancer cell line HT29 and its relationship with apoptosis. Although it is known that SBE13 has a cytotoxic effect in many cancer types, to the best of our knowledge, there is no study on HT29 cells.

## Material and Methods

### *Cell lines and cell culture*

The human colorectal cancer cell line HT-29 (ATCC, HTB-38) obtained from ATCC (American Type Culture Collection) was used in the study. The HT29 cell line was cultured in DMEM (Gibco Thermo Fisher Scientific) supplemented with 50 U/mL penicillin/streptomycin (SigmaAldrich) and 10% fetal bovine serum (Sigma-Aldrich). The cells were then incubated at 37°C and in an environment containing 5% $CO_2$.

### *Cell viability assay*

SBE13 (Cayman) stock solution was prepared in DMSO to a final concentration of 10 mM. Growing cells were seeded in a 96-well plate at $1X10^4$ per well. Cells were treated at different concentrations (1, 5, 10, 25, 50, and 100 µM) for 48 hours. After 48 hours of incubation, cell viability was measured using XTT (Roche Diagnostic) colorimetric method. All experimental steps were performed in triplicate. Cell viability was calculated as a percentage of viable cell amount compared to control cells. The $IC_{50}$ (half-maximum inhibitory concentration) of SBE13 in HT-29 cells was calculated using Graph Prism 7 software.

### *Annexin V binding assay*

Initially, prepared HT-29 cells were placed in six-well plates. After observing cell attachment, cells were exposed to SBE13 at the determined $IC_{50}$ concentration. After 48 h of incubation, cells were harvested and incubated with Muse™ Annexin V & Dead Cell kit reagent. The protocol recommended by the manufacturer was used in the procedure. Using Annexin V and/or 7-AAD positivity on the Cell Analyzer (Muse, Millipore), four different populations were monitored: live, early apoptotic, late apoptotic, and dead.

### *Statistical analysis*

All experiments were performed in triplicate and the data obtained were expressed as mean ± standard deviation. The post hoc Dunn test or the Mann-Whitney test as well as the Kruskal-Wallis ANOVA test were used as appropriate to compare the variables measured as a result of the application of SBE13 with the control. Statistical difference was considered significant when $P<0.05$.

## Results

**Targeting of PLK1 activity via SBE13 and cell viability effects on HT-29 cells**

The cytotoxic effect of the PLK1 inhibitor SBE13 was examined in HT-29 human colon cancer cells. Cells were exposed to different doses of SBE13 (1-100 µM) for 48 h. The antiproliferative effect that may occur as a result of this procedure was examined using the XTT colorimetric method. The cell viability results obtained are given in Figure 1 and SBE13 was shown to have a dose-dependent inhibitory effect at 48 h compared to the control group ($P < 0.05$).

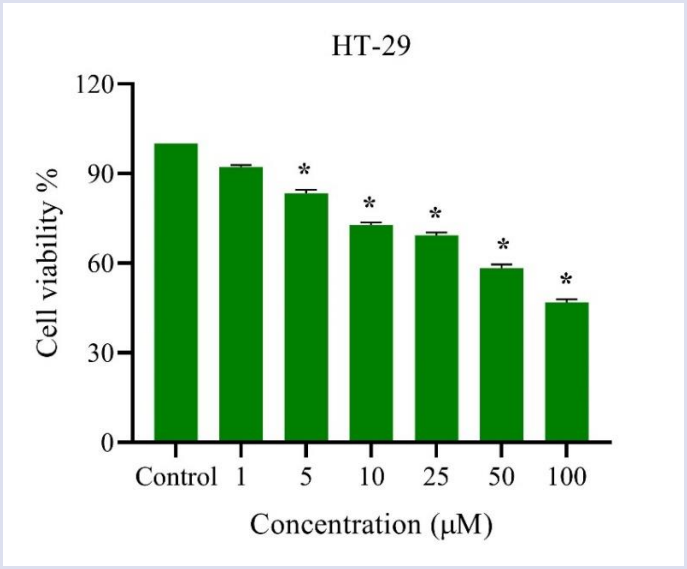


**Figure 1.** The percentage of viability of HT-29 cells, which were treated with SBE13 at different concentrations (1, 5, 10, 25, 50, and 100 µM) for 48 h, was measured by XTT colorimetric assay, and the average values were given in the table after three replications.
*Results obtained are significantly different when compared to the viability of the Control group cells (P<0.05).

The effect of SBE13 was evaluated in HT-29 cells for 48 h and the $IC_{50}$ value was calculated to be 11.79 µM, and this concentration was used in the later stages of the study.

### *SBE13 induces apoptosis in HT-29 cells*

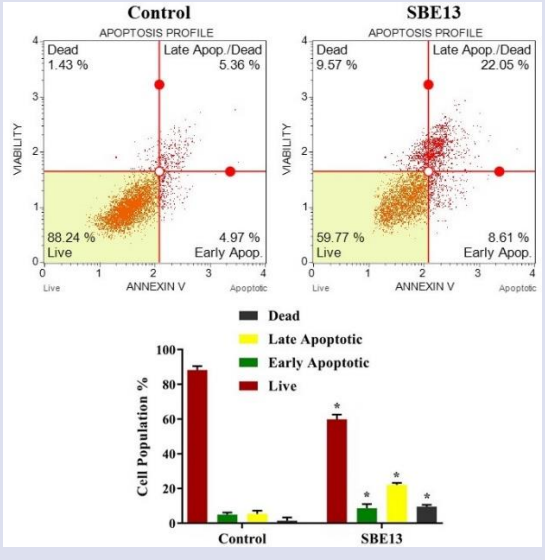


**Figure 2.** The cell population ratio resulting from apoptosis in HT-29 cells after 48 h of exposure to SBE13 at an $IC_{50}$ dose is determined by the Annexin V assay. All experiments were performed in triplicate.

The effect of SBE13 on apoptosis in HT-29 colon cancer cells was evaluated by flow cytometry with the aid of Annexin V binding assays. According to the results obtained, the data obtained as a result of exposure of HT29 cells to SBE13 supported that it significantly increased the early and late apoptotic cell population compared to control cells ($P < 0.05$). *Significant reduction was observed compared to the cell population ratio of control cells ($P < 0.05$).

As seen in Figure 2, the percentage of early and late apoptotic cell population in cells not exposed to SBE13 (4.97±1.15%, 5.36±1.87%, respectively) was significantly 8.61±2.37% and 22.05±1. 19%, respectively, in the SBE13 administered group ($P < 0.05$).

## Discussion

This study was conducted to evaluate the cytotoxic and apoptotic effects of SBE13, known to be a PLK1 inhibitor, on HT29 cells representing one of the most common cancers, colon adenocarcinoma. In the study, it was shown that SBE13 has a serious cytotoxic effect on HT29 cells. At another stage of the study, SBE13 was shown to induce apoptosis. When the results obtained are examined in general, it has been shown that SBE13 has significant effects on HT29 and has the potential for anticancer activity.

PLK1 has an important role in regulating the proliferation process of cells. There is ample evidence to suggest that overexpression of PLK1 has a profound effect on the occurrence of certain types of cancer in humans. Based on this information, it

has been shown that PLK1 inhibition may have an important role in cancer therapy [11]. Inhibition of PLK1 in many different cancer cells has been shown to cause death in cancer cells through apoptosis and has become an important anticancer drug target. For this reason, many studies have reported the anticancer activities of PLK1 inhibitors in different cancer types. [12]. Although monotherapeutic use is promising, combination treatments have also been found to be effective in many studies [13]. Many PLK1 inhibitors such as ON01910.Na, RO3280, HMN-214, NMS-P937, and TKM-080301 have been examined in the literature for their anticancer activity [9,14,15]. One of the PLK1 inhibitors is SBE13. There are studies on the anticancer activity of SBE13. In one of these studies, SBE13 was shown to have anticancer activity in the breast cancer cell line MDA-MB-231 [16]. In another study, the effect of TAK-960, which is a PLK1 inhibitor, against solid malignancies such as A2780, SW620, K562, and HT29 was examined. It has been reported that TAK-960, a PLK1 inhibitor, has a serious antiproliferative effect in all cancer cells studied [17]. In another study, Lange et al. [18], demonstrated the effects of enzastaurin, an inhibitor of SBE13 and PCKβ, on various tumor cells such as HeLa, MCF-7, hTERT-RPE1, HCT116p53+/+, and HCT116p53-/-. In this study, synergistic effects of SBE13 and enzastaurin were demonstrated on cancer cell lines other than hTERT-RPE1. Also, the combination of SBE13 and enzastaurin has been reported to increase apoptosis in HeLa cells compared to enzastaurin alone.

In the light of all these data, it is thought that PLK1 has a serious effect on cancer development, prognosis, and progression. Inhibition of PLK1 appears to play an important role in its anticancer activity, as many studies have shown. It is understood that apoptosis plays an active role in the development of these cytotoxic effects. In our study, the effects of another PLK1 inhibitor, SBE13, on HT29, a human colon adenocarcinoma, were investigated. In our in vitro study, it was determined that SBE13 caused severe cytotoxicity in HT29 cells. In the next stage of the study, the efficacy of apoptosis in this cytotoxicity was determined.

## Conclusion

When the literature is examined collectively, it is seen that PLK1 has a very important role in the development and prognosis of many human cancers. It is reported that specific inhibition of PLK1 reduces the development of cancer cells and contributes positively to the prognosis. In our study, it was shown that SBE13 administration in HT29 cells caused a serious cytotoxic effect by inducing apoptosis. In conclusion, it is predicted that SBE13, a PLK1 inhibitor, may be a promising anticancer agent for colon cancer treatment. However, more studies are needed to elucidate the potential use of SBE13 in the treatment of colon cancer.

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