ISSN 2395-650X

International Journal of Life Sciences Biotechnology Pharma Sciences

E-mail: editorijlbps@gmail.com editor@ijlbps.org

International Journal of Life Sciences Biotechnology Pharma Sciences

ISSN 2395-650X

Volume 10 Issue4Dec2021

Analysis of SGM 597, a flurbiprofen derivative, on breast cancer cell lines: effects on cell proliferation and apoptosis

Ushasri,.P.Mary, K.Manisha,Pokala sumanasri

ABSTRACT

Purpose and Background: Breast cancer is a growing health concern, particularly among females. Researchers are concentrating on finding new medications that help combat breast cancer. There is a striking degree of biological similarity between breast cancer and prostate cancer. Accordingly, the 4-(4-chlorophenyl)-3-(1-(2-fluoro-[1,1'-biphenyl]-4-yl)ethyl), 5-((4-fluorobenzyl)thio)Research using the MCF-7 and MCF-10A breast cancer and mammary epithelial cell lines examined the effects of the chemical SGK597, which inhibits cell growth in prostate cancer.

Methods: To find out if SGK597 was cytotoxic or viable in MCF-7 and MCF10-A cell lines, the WST-8 technique was used. We used the JC-1 test to find out whether the potential across the mitochondrial membrane changed. Western blot analysis was used to evaluate the amounts of apoptosis-associated proteins, including Bax, Bcl-2, and c-PARP.

The IC50 values for MCF-7 were 28.74 μM and 17.28 μM after 24 hours of incubation with SGK597, while for MCF-10A, they were 65.9 μM and 50.5 μM, respectively. While MCF-10A cells did not exhibit a trend toward depolarization of the mitochondrial membrane potential in response to increasing concentrations of SGK597, MCF-7 cells did. No rise in the Bax/Bcl-2 ratio or c-PARP expression level was seen in western blot tests, suggesting the absence of apoptosis.

Results showed that SGK597 inhibited the growth of MCF-7 cells. These findings suggest that SGK597 might be an effective anticancer drug.

Definitions: thioether, triazole, apoptosis, flurbiprofen, breast cancer

INTRODUCTION

Among female cancers, breast cancer is by far the most prevalent. Breast cancer affects over 1.5 million people globally annually. Age, a woman's family medical history, and the BRCA1 and BRCA2 gene abnormalities that are believed to increase the likelihood of breast cancer are the primary risk factors for this disease in women (Becker, 2015; Sun et al., 2017). There is an ongoing need to discover new anticancer medications due to the high rate of cancer recurrence and the severe adverse effects of current chemo treatments (Ali et al., 2012). Meegan and O'Boyle (2019) report that novel anticancer medications target proteins with aberrant expression in cancer cells, in contrast to classic chemotherapeutic agents that primarily target cancer cells' DNA.

We know that chronic inflammation and cancer go hand in hand. So, anti-inflammatory medications might have a role in cancer prevention and therapy. Arachidonic acid is released from cellular phospholipids by the enzyme phospholipase A2 in response to inflammatory stimuli.

SAMSKRUTI COLLEGE

Arachidonic acid may be converted into prostaglandins (PGs) by two different types of the cyclooxygenase (COX) enzyme: COX-1 and COX-2. Inflammation may be facilitated by PGs. Research using cell lines from patients with breast cancer has shown that COX-2 overexpression is a key factor in the development of this disease. As a result of their capacity to inhibit the COX enzyme, non-steroidal anti-inflammatory medications (NSAIDs) may prevent the production of COX products. ("±") (R, S)One example of an NSAID is flurbiprofen, which is not selective for COX inhibitors. Flrubiprofen stops the formation of PG by blocking the enzyme PG synthetase's COX activity. That was in 1983 (McCormick & Moon). Researchers have shown that flurbiprofen and its structural variants have anticancer properties (Aydın et al., 2013; Çıkla et al., 2013). Adding flurbiprofen to chemotherapy improved therapeutic response in individuals with metastatic breast cancer (Powles, Alexander, & Millar, 1978). Furthermore, it has been claimed that triazole (Küçükgüzel & Çıkla-Süzgün, 2015; Çıkla-Süzgün & Küçükgüzel, 2021) and thioether derivatives (Çoruh, Çevik, Yelekçi, Djikic, & Küçükgüzel, 2017; Birgül et al., 2020; Han & Küçükgüzel, 2022) are capable of inhibiting cancer. Chemically, the SGK597 molecule is 4-(4-chlorophenyl) flurbiprofen triazole-thioether, as shown in Figure 1.-3-(1-(2-fluoro- [1,1'-biphenyl]-4yl)ethyl), 5-((4-fluorobenzyl)thio)4-H-1,2,4-triazole chemical formula.

The SGK597 molecule primarily targets MetAP-2, a cytoplasmic methionine aminopeptidase found in mammalian cells that is known to be highly expressed in cancer cells. After 24 hours of incubation, the chemical SGK597 was shown to reduce the proliferation of androgen receptor-positive prostate cancer cells LNCaP, PC-3, and DU-145 cells, respectively, with IC50 values of 27.1, 6.9, and 106.7 µM (Yılmaz et al., 2020).

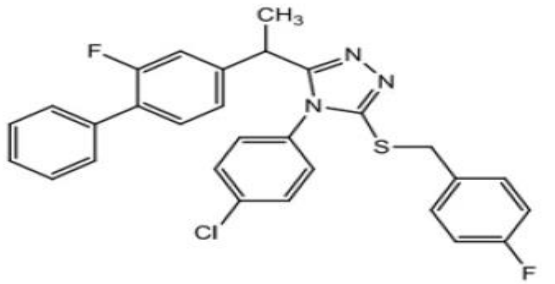

Figure 1. Chemical structure of Flurbiprofen derivative SGK597 (Yılmaz et al., 2020).

A large number of men will get prostate cancer at some point in their lives. Cancers of the breast and prostate have many common biological features. Patients with a personal or familial history of breast cancer who have the BRCA1 or BRCA2 mutations are more likely to develop prostate cancer, according to the available data. Prior research has also shown a correlation between a first-degree relative's breast cancer diagnosis and prostate cancer (Ren et al., 2019).

New approaches to treating prostate and breast cancer have emerged as researchers have delved deeper into the diseases' underlying mechanisms (Risbridger, Davis, Birrell, & Tilley, 2010). Green tea's high concentration of epigallocatechin gallate slowed the proliferation and reduced the size of human breast and prostate cancer cells in an in vivo experiment (Liao, Umekita, Guo, Kokontis, & Hiipakka, 1995). It is reported that carbaryamidotriazole has entered phase III clinical trials in cancer patients. The molecule was shown to selectively block the migration of breast and prostate cancer in vitro (Bradke, Hall, Carper, & Plopper, 2008). Imai, Yokoe, Tsubuki, & Takahashi (2019) found that some cinnamic acid derivatives have an inhibitory effect on the proliferation of breast and prostate cancer cells via causing apoptosis. A different in vitro investigation found that a number of tamoxifen-estrogen-artemisinin hybrid compounds had anticancer effects in human breast and prostate cancer cell lines (Fröhlich et al., 2020). Consequently, it is reasonable to assume that chemicals with anticancer effects in prostate cancer may also work in breast cancer.

This study's objective is to identify the cytotoxic and apoptotic effects of the SGK597 compound in MCF-7 breast cancer and MCF-10A mammary epithelial cell lines using the WST-8 test, JC-1 mitochondrial membrane potential test, and Western blotting. The concentrations tested were 0, 10, 25, 50, 75, and 100 µM, and the durations tested were 24 and 48 hours.

MATERIAL AND METHODS

The cultivation of cells

In an incubator with 5% CO2, MCF-7 breast cancer cells and MCF-10A mammary epithelial cells were cultured in different media at 37 °C. The first medium contained 10% FBS and 1% Pen/Strep, while the second contained 5% horse serum, 0.02% EGF, 0.05% hydrocortisone, 0.01% cholera toxin, 0.1% insulin, and 1% Pen/Strep. Three times weekly, the medium was switched.

Methods for Making an SGK597 Base Solution

To make a 96 mM stock solution, 50 mg of SGG597 (which was provided as a white powder) was dissolved

ISSN 2395-650X
Volume 10 Issue4Dec2021

in 1 mL of DMSO. The stock was then administered to cells at lesser quantities colourimetric WST-8 assay

Using a CCK-8 kit that is based on the WST-8 colorimetric change, the effects of SGK597 on cell viability and cytotoxicity in MCF-7 and MCF-10A cell lines were examined. The treatment group was exposed to varying doses of SGK597 for 24 and 48 hours after 1500 cells/well were seeded onto 96-well plates. Then, cells were counted using the CCK-8 kit (KTC011001, Abbkine) as directed by the manufacturer. Four hours later, absorbance at 450 nm was detected using a microplate reader (Synergy H1, BioTek Instruments Inc., USA) manufactured by BioTek.

JC-1 Assay for Mitochondrial Potential

Two cell lines, MCF-7 and MCF-10A, were tested for mitochondrial membrane potential using the JC-1 assay. Following the manufacturer's instructions, cells were seeded on a 96-well black opaque plate and treated with SGK597 at various doses (10, 25, 50, 75, 100 µM) for 48 hours. They were then stained with JC-1 dye (JC-1 Mitochondrial Membrane Assay Kit, 10009172, Cayman Chemical). Mega Tiber, Kocyigit Sevinc, Kilinc, and Orun (2019) used the green/red fluorescence ratio to assess cell apoptosis.

For each sample, 8 µL of protease inhibitor cocktail, 2 µL of NaF, and 190 µL of RIPA lysis buffer (RIPA Lysis Buffer System, sc-24948A, Santa Cruz) were used to lyse the MCF-7 cell pellets for Western blotting. Fifty micrograms of protein from every sample was subjected to 2 hours of SDS-PAGE running at 150 V, with 2 hours of transfer to the membrane at 25 V. A 5% BSA block was applied. The membranes were exposed to β-actin, Bcl-2, Bax, and c-PARP primary and secondary antibodies dissolved in 1X TBS-T with 1% BSA. A chemiluminescent substrate solution (WesternBright ECL HRP Substrate, Advansta) was used for the detection. The Biostep Celvin chemiluminescence imaging device and TotalLab 1D software were used for protein quantitation.

Data analysis

After doing one-way ANOVA analysis, Dunnett's post hoc tests were used to analyze the findings of the WST-8 colorimetric test and the JC-1 mitochondrial membrane potential. After running the western blot data through Kruskal Wallis, we used Dunn's post hoc tests to see if the data held up.

The statistical tests were carried out with the help of GraphPad Prism (version 8.0.1, GraphPad Software, CA, USA). For $p < 0.05$, the degree of significance was deemed acceptable.

RESULTS

Persistence of cells and harm to cells

Figure 2 shows the absorbance values for MCF-7 cells and Figure 3 shows the absorbance values for MCF-10A cells after the WST-8 test. Table 1 displays the IC50 values that were established by analyzing the absorbance values obtained from the WST-8 test for incubation of MCF-7 and MCF-10A cells with SGK597 at various doses and durations.

Cell death

An important role that mitochondria play in cell death has long been recognized (Ly, Grubb, & Lawen, 2003). Mitochondrial permeability transition pore opening during cell death

Table 1. IC_{50} values in µM for MCF-7 and MCF-10A cells after 24 and 48 hours of incubation with SGK597 compound.

SGK597 (µM)

Incubation period	24h	48h
MCF-7	28.74	17.28
MCF-10A	65.9	50.5

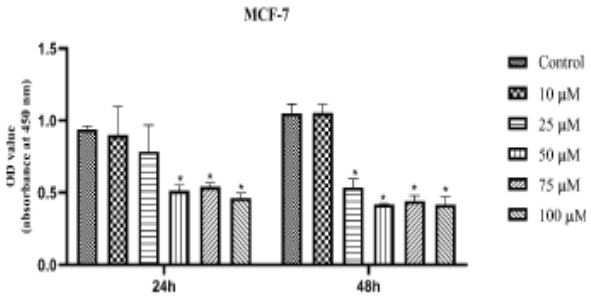

Figure 2. The absorbance values of MCF-7 cells were shown in a bar chart with standard deviation error bars after 24 and 48 hours of incubation with SGK597 (n = 3). The concentration-dependent variations in absorbance were statistically significant. Based on post-hoc comparisons using Dunnett's test, there was a significant difference (*$p < 0.05$) between the control group and the groups treated with 50, 75, 100 µM at 24 hours of incubation and 25, 50, 75, 100 µM at 48 hours of incubation. O.D. stands for optical density.

International Journal of Life Sciences Biotechnology Pharma Sciences
ISSN 2395-650X
Volume 10 Issue4Dec2021

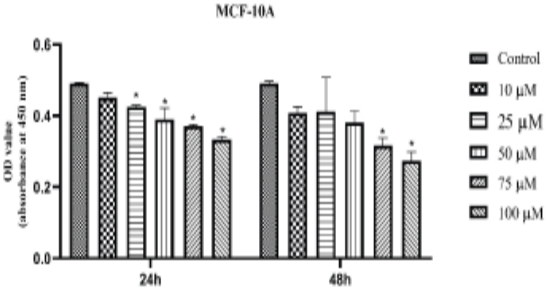

Figure 3. The absorbance values of MCF-10A cells after 24 and 48 hours of incubation with SGK597 are shown in a bar chart along with the standard deviation error bars. The sample size was 3. The concentration-dependent variations in absorbance were statistically significant. The use of Dunnett's test for post-hoc comparisons revealed a significant difference from the control group in the mean absorbance score for 25, 50, 75, and 100 µM after 24 hours of incubation, as well as for 75 and 100 µM after 48 hours of incubation (*p < 0.05). O.D. stands for optical density.

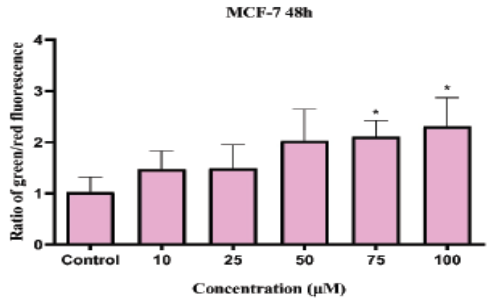

Figure 4. Using a standard deviation error bar as a measure, this bar chart displays the ratio of green to red fluorescence for both the control and experimental groups of MCF-7 cells after 48 hours of incubation with SGK597 (n = 3).

The ratio of green to red fluorescence was significantly different for the doses in MCF-7 cells (p = 0.036). According to post-hoc comparisons conducted using Dunnett's test, there was a significant difference (*p < 0.05) in the control group's mean score of the green/red fluorescence ratio for 75 µM and 100 µM with the electrochemical gradient disappearing and mitochondrial. We anticipate a membrane potential. At 48 hours of incubation with varying dosages of SGK597, Figure 4 shows the green/red fluorescence ratios obtained from the JC-1 mitochondrial membrane potential test on MCF-7 cells and Figure 5 shows the same findings on MCF-10A cells. Apoptotic cells fluoresced green, whereas healthy cells fluoresced red.

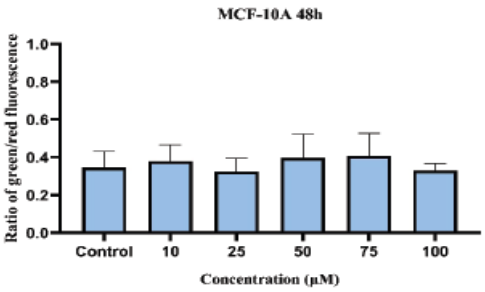

Figure 5. Figure 3 shows the green/red fluorescence ratio in MCF-10A cells after 48 hours of incubation with Sgk597. The error bars represent the standard deviation for the control and experimental groups. The ratio of green to red fluorescence did not change significantly among doses for MCF-10A cells (p = 0.822).

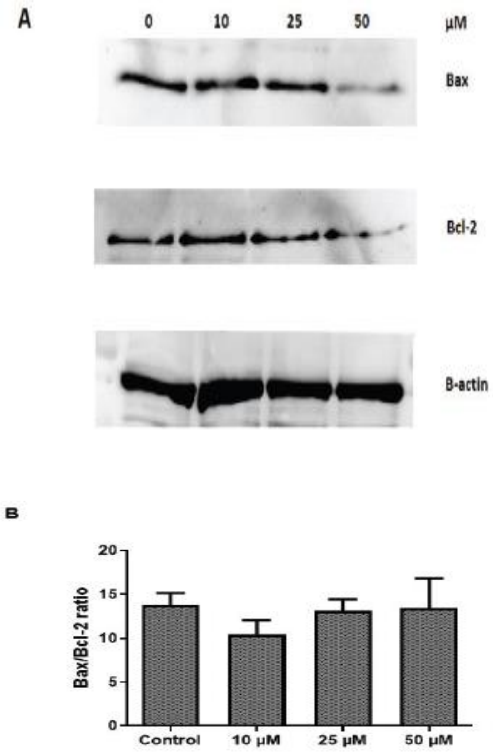

Figure 6. The outcomes of the Bax/Bcl-2 Western blot. (A) Expression of Bax/Bcl-2 protein using β-actin as a standard in MCF-7 cells after 48 hours of incubation with SGK597. The standard deviation error bars are shown on the bar chart showing band intensities that have been adjusted to β-actin (n = 2). Statistical

analysis revealed no significant variation in the levels of Bax/Bcl-2 protein expression (p = 0.495).

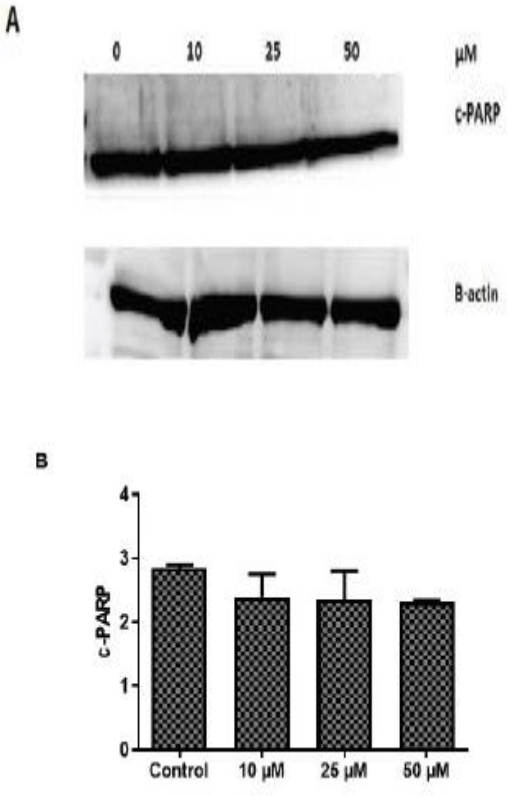

Figure 7. Western blotting analysis of c-PARP. (A) Expression of c-PARP protein using β-actin as a standard in MCF-7 cells after 48 hours of incubation with SGK597. The band intensities, normalized to β-actin, are shown in a bar chart with standard deviation error bars, for a sample size of 2. The levels of c-PARP protein expression were not significantly different (p = 0.343).

DISCUSSION

The possibility of a cytotoxic action against prostate cancer cells was earlier found in the flurbiprofen derivative SNK597 (Yılmaz et al., 2020). Evidence from earlier research suggests that drugs that have shown promise in treating prostate cancer may have a similar impact on breast cancer. Based on this, we investigated if SGK597 was cytotoxic to breast cancer cell lines. After incubating MCF-7 and MCF-10A cell lines with SGK597 for 24 and 48 hours, the IC50 values were determined. After incubating SGK597 for 24 hours, the IC50 value found in prostate cancer cells by Dr. Kücükgüzel and colleagues was 27.1 μM.

It is suggested that there could be a connection between the two cancer types since this number is near the value found for MCF-7, which is 28.74 μM. A far higher IC50 value was observed in MCF-10A epithelial cells. Accordingly, it seems that MCF-10A will be unaffected by SGK597 treatment throughout the same incubation time as MCF-7. All cells that are dead or in the early or late stages of apoptosis, when cellular processes are halted, determine the IC50 value. The WST-8 test cannot detect apoptosis, hence. Nonetheless, it provides some insight into cell viability and metabolic activity.

Depolarization of the mitochondrial membrane potential showed that apoptosis was induced, although the Bax/Bcl-2 ratio and c-PARP level did not. It is anticipated that cells going through apoptosis would have an elevated c-PARP expression level and a Bax/Bcl-2 ratio. The findings provide credence to the theory that SGK597 does not induce cell death in MCF-7 cells. Along these lines, the JC-1 mitochondrial membrane potential test showed depolarization, which implies that In addition to its ability to inhibit proliferation, SGK597 may also stimulate cell death, suggesting that it may have dual uses. It is necessary to repeat tests at least three times when using Western blotting for quantitative assessment since it is not particularly sensitive. To precisely identify apoptotic cell death, more testing is required.

The chemical SGK597 was created as a good MetAP-2 inhibitor, therefore studies with various proteins may reveal that the medication is effective on multiple pathways. The effect of SGK597 on MetAP-2 levels in the MCF-7 cell line should also be confirmed experimentally. MetAPs are proteases that clean up the amino terminal of freshly made proteins by eliminating methionine. It is known that MetAP-1 and MetAP-2 exhibit MetAP activity in eukaryotic organisms. According to recent research, MetAP-2 is involved in angiogenesis, which is crucial for the development of several tumor types. Speculation has it that MetAP-2 acts as a cancer gene.

Thus, by lowering its concentration, this enzyme may be used to combat cancer cells. For the treatment of metastatic breast cancer, the TNP-470 compound has reportedly entered human clinical trials (Selvakumar et al., 2006; Selvakumar et al., 2009). Additionally, it has been found to inhibit the growth of MDA-MB-231 triplenegative breast cancer cells (Yamaoka et al., 1993).

After flurbiprofen administration, a different research found that the HeLa and HepG2 human cancer cell

lines exhibited cytotoxic, genotoxic, and apoptotic effects via the intracellular route (Bakır et al., 2021). Additionally, flurbiprofen was found to inhibit the growth of different tumor cells in a dose-dependent manner when tested against tumor cell lines derived from medulloblastoma and glioblastoma multiform (King & Khalili, 2001). Additionally, our investigation confirmed the proliferation-suppressing effects of SGK597. Nevertheless, in order to prove apoptosis, this finding must be backed by other approaches.

Results from research examining the impact of nonsteroidal anti-inflammatory drugs (NSAIDs) on breast cancer have been inconsistent. We still don't know how NSAIDs affect the progression of breast cancer, so it's hard to say whether the variations are a result of medication design or something else entirely.

Finally, both breast and prostate cancer cells were shown to exhibit cell proliferation inhibitory characteristics when exposed to the SGK597 molecule, a derivative of flurbiprofen. Future investigations will build upon this basic study.

REFERENCES

• Ali, R., Mirza, Z., Ashraf, G. M., Kamal, M. A., Ansari, S. A., Damanhouri, G. A., … Sheikh, I. A. (2012). New anticancer agents: recent developments in tumor therapy. *Anticancer Research, 32*(7), 2999–3005.

• Aydın, S., Kaushik-Basu, N., Arora, P., Basu, A., Nichols, D. B., Talele, T. T., … Kucukguzel, Ş. G. (2013). Microwave assisted synthesis of some novel Flurbiprofen hydrazidehydrazones as anti-HCV NS5B and anticancer agents. *Marmara Pharmaceutical Journal, 17*, 26- 34.

• Bakır, E., Cal, T., Aydın Dilsiz, S., Canpınar, H., Eken, A., & Undeğer Bucurgat, U. (2021). Assessment of the cytotoxic, genotoxic, and apoptotic potential of flurbiprofen in HeLa and HepG2 celllines. *Journal of biochemical and molecular toxicology*, 35(6), 1–11. https://doi.org/10.1002/jbt.22770

• Becker S. (2015). A historic and scientific review of breast cancer: The next global healthcare challenge. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics, 131 Suppl 1*, S36–S39. https:// doi.org/10.1016/j.ijgo.2015.03.015

• Birgul, K., Yıldırım, Y., Karasulu, H. Y., Karasulu, E., Uba, A. I., Yelekci, K., … Kucukguzel, Ş. G. (2020). Synthesis, molecular modeling, in vivo study and anticancer activity against prostate cancer of (+) (S)-naproxen derivatives. *European journal of medicinal chemistry, 208*, 112841. https://doi.org/10.1016/j.ejmech.2020.112841

• Bradke, T. M., Hall, C., Carper, S. W., & Plopper, G. E. (2008). Phenylboronic acid selectively inhibits human prostate and breast cancer cell migration and decreases viability. *Cell adhesion & migration, 2*(3), 153–160. https://doi.org/10.4161/cam.2.3.6484

• Cıkla, P., Tatar, E., Kucukguzel, İ., Şahin, F., Yurdakul, D., Basu, A., Kucukguzel, Ş. G. (2013). Synthesis and characterization of flurbiprofen hydrazide derivatives as potential anti-HCV, anticancer and antimicrobial agents. *Medicinal Chemistry Research, 22*(12), 5685-5999. https://doi.org/10.1007/s00044-013-0550-3

• Cıkla-Suzgun, P., & Kucukguzel, Ş. G. (2021). Recent Progress on Apoptotic Activity of Triazoles. *Current drug targets, 22*(16), 1844– 1900. https://doi.org/10.2174/1389450122666210208181128

• Coruh, I., Cevik, O., Yelekci, K., Djikic, T., & Kucukguzel, Ş. G. (2018). Synthesis, anticancer activity, and molecular modeling of etodolac-thioether derivatives as potent methionine aminopeptidase (type II) inhibitors. *Archiv der Pharmazie, 351*(3-4), e1700195. https://doi.org/10.1002/ardp.201700195

• Frohlich, T., Mai, C., Bogautdinov, R. P., Morozkina, S. N., Shavva, A. G., Friedrich, O., … Tsogoeva, S. B. (2020). Synthesis of Tamoxifen-Artemisinin and Estrogen-Artemisinin Hybrids Highly Potent Against Breast and Prostate Cancer. *ChemMedChem, 15*(15), 1473–1479. https://doi.org/10.1002/cmdc.202000174

• Han, M. İ., & Kucukguzel, Ş. G. (2022). Thioethers: An Overview. *Current drug targets, 23*(2), 170–219. https://doi.org/10.2174/1389450122666210614121237