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In vitro evaluation of the cytotoxic effects (IC50) of climbazole-alcohol compound on various human cancer cell lines

Climbazole-alkol bileşiğinin farklı insan kanser hücre hatları üzerindeki sitotoksik etkisinin (IC50) *in vitro* değerlendirilmesi

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ABSTRACT

Objective: Cancer is one of the most significant health problems in both developed and developing countries today. Most of the currently available anticancer drugs can cause severe side effects and toxicities, which negatively affect patients' quality of life. Therefore, the development of safer and more selective anticancer agents remains one of the primary goals of contemporary oncology research. In recent years, the cytotoxic and antiproliferative effects of various pharmacological agents on different cancer cell lines have been extensively studied. This study aimed to investigate the potential anticancer effects of the Climbazole (CBZ) derivative, Climbazole-alcohol, on different human-derived cancer cell lines (HT-29, MKN28, A549, MDA-MB-231) and a normal control cell line (HEK293) under in vitro conditions.

Methods: CBZ was reduced to Climbazole-alcohol according to previously reported procedures, and the structure was confirmed by ^{1}H-NMR spectroscopy. Human cancer cell lines (HT-29, MKN28, A549, MDA-

ÖZET

Amaç: Kanser, günümüzde hem gelişmiş hem de gelişmekte olan ülkelerde en önemli sağlık sorunlarından biridir. Mevcut kanser ilaçlarının çoğu, hastaların yaşam kalitesini olumsuz etkileyen ciddi yan etkilere ve toksisitelere neden olabilir. Bu nedenle, daha güvenli ve daha seçici kanser önleyici ajanların geliştirilmesi, çağdaş onkoloji araştırmalarının temel hedeflerinden biri olmaya devam etmektedir. Son yıllarda, çeşitli farmakolojik ajanların farklı kanser hücre hatları üzerindeki sitotoksik ve antiproliferatif etkileri kapsamlı bir şekilde incelenmiştir. Bu çalışma, Climbazole (CBZ) türevi olan Climbazole-alkolün, farklı insan kaynaklı kanser hücre hatları (HT-29, MKN28, A549, MDA-MB-231) ve normal kontrol hücre hattı (HEK293) üzerindeki potansiyel kanser önleyici etkilerini in vitro koşullarda araştırmayı amaçlamaktadır.

Yöntem: CBZ, literatürde bildirilen yöntemlere göre indirgeme reaksiyonu ile Climbazole-alkol formuna dönüştürülmüş, bileşiğin yapısı ^{1}H-NMR analiziyle doğrulanmıştır. Hücre kültürü çalışmaları, ATCC kaynaklı

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MB-231) and the normal HEK293 cell line were cultured under standard conditions at 37°C in a humidified atmosphere containing 5% CO_2. Cells were treated with various concentrations of Climbazole-alcohol for 72 hours. Cytotoxicity was assessed using the MTT assay, and IC_{50} values were determined using GraphPad Prism software.

Results: Climbazole-alcohol induced a mild decrease in cell viability in all tested cell lines but did not exhibit a significant cytotoxic effect. The calculated IC_{50} values were 179.2 µM for HT-29, 168.1 µM for MKN28, 164.1 µM for A549, 158.6 µM for MDA-MB-231, and 169.8 µM for HEK293. No statistically significant difference was observed between cancer and normal cell lines ($p>0.05$).

Conclusion: Climbazole-alcohol did not display selective anticancer activity within the tested concentration range. The findings indicate that this compound lacks cancer-specific cytotoxicity, suggesting limited potential as an anticancer agent. Future studies should focus on structural modifications or combination therapies to enhance its anticancer properties.

Key Words: Cancer cell lines, Climbazole-alcohol, Cytotoxicity, HEK293, MTT assay

HT-29, MKN28, A549, MDA-MB-231 ve HEK293 hücre hatları üzerinde yürütülmüştür. Hücreler 37°C'de, %5 CO_2 atmosferinde uygun kültür ortamlarında çoğaltılmış ve 72 saat boyunca farklı Climbazole-alkol konsantrasyonlarına maruz bırakılmıştır. Sitotoksisite, MTT hücre canlılık testi ile değerlendirilmiş ve IC_{50} değerleri GraphPad Prism yazılımı kullanılarak hesaplanmıştır.

Bulgular: Climbazole-alkol, test edilen tüm hücre hatlarında düşük düzeyde hücre canlılığı azalmasına neden olmuş, ancak belirgin bir sitotoksik etki göstermemiştir. IC_{50} değerleri sırasıyla HT-29 için 179,2 µM, MKN28 için 168,1 µM, A549 için 164,1 µM, MDA-MB-231 için 158,6 µM ve HEK293 için 169,8 µM olarak hesaplanmıştır. Kanser hücre hatları ile kontrol hattı arasında anlamlı bir fark gözlenmemiştir ($p>0.05$).

Sonuç: Climbazole-alkol bileşiği, incelenen konsantrasyon aralıklarında seçici antikanser aktivite göstermemiştir. Bulgular, bu bileşiğin kanser hücrelerine özgü toksisite oluşturmadığını ve dolayısıyla antikanser ajan potansiyelinin sınırlı olduğunu ortaya koymaktadır. Gelecekte yapılacak çalışmalar, bileşiğin farklı türevlerinin veya kombinasyonlarının antikanser etkinlik açısından değerlendirilmesi yönünde olmalıdır.

Anahtar Kelimeler: Kanser hücre hatları, Klimbazol-alkol, Sitotoksisite, HEK293, MTT

INTRODUCTION

Cancer is one of the most significant health problems in both developed and developing countries today. The increasing global incidence and mortality rates of cancer have made it essential to develop new therapeutic approaches and chemotherapeutic agents. The fundamental characteristics of cancer include uncontrolled cell proliferation, evasion of apoptosis, the ability to metastasize, and the promotion of angiogenesis (1). These processes contribute to the emergence of various types of cancer in different organs and tissues.

Cell lines such as lung cancer (A549), breast cancer (MDA-MB-231), gastric cancer (MKN28), and colorectal cancer (HT-29) are widely used *in vitro* models for understanding cancer biology and developing new therapeutic strategies(1-3). Most of the currently available anticancer drugs can cause severe side effects and toxicities, which negatively affect patients' quality of life. Therefore, the development of safer and more selective anticancer agents remains one of the primary goals of contemporary oncology research (4, 5).

In recent years, the cytotoxic and antiproliferative effects of various pharmacological agents on different cancer cell lines have been extensively studied (6). Cell lines used in cancer research are of great importance for elucidating the molecular mechanisms of the disease, evaluating the cytotoxic and antiproliferative effects of drugs, and investigating resistance mechanisms in cancer cells. Particularly, the A549 lung cancer cell line, MDA-MB-231 triple-negative breast cancer cell line, MKN28 gastric cancer cell line, and HT-29 colorectal cancer cell line are among the most commonly studied human cancer cell lines in the literature (7-9).

In addition to these, normal human embryonic kidney cell line (HEK293) is often used as a control to assess the selectivity and specificity of toxic effects toward cancer cells. The use of cancer cell lines plays a crucial role in developing new anticancer agents and improving the efficacy of current treatment strategies (10).

Lung cancer is one of the leading causes of cancer-related deaths worldwide. The A549 cell line, derived from human lung adenocarcinoma, is one of the *in vitro* models frequently used to study the anticancer effects of cytotoxic agents and natural compounds (11, 12). Breast cancer is the most common cancer among women, and the MDA-MB-231 cell line is widely used as a model for triple-negative breast cancer (9, 13, 14).

Gastric cancer is one of the most common and fatal tumors of the gastrointestinal system. The MKN28, NCI-N87, and AGS cell lines are frequently used in gastric cancer research. Colorectal cancer is among the leading causes of cancer-related deaths worldwide, and the HT-29 cell line is widely used as an *in vitro* model for this cancer type (15-17).

Normal cell lines also play a critical role in toxicity evaluations alongside cancer cell lines. The HEK293 cell line, derived from human embryonic kidney cells, is widely used to represent normal cell physiology. The absence of toxicity in normal cell lines such as HEK293 when exposed to test compounds indicates selective anticancer potential (18).

In recent years, the development of targeted and selective anticancer agents has gained great importance. Compounds that exhibit high toxicity toward cancer cell lines but show no toxicity toward normal cells are considered potential anticancer agents (19). Developing such agents would enable the preservation of healthy tissues and minimize side effects during cancer treatment. Numerous studies have demonstrated that various natural and synthetic compounds exert significant cytotoxic and antiproliferative effects on cancer cells while remaining non-toxic to normal cells (15, 18).

In this study, the anticancer potential of Climbazole (CBZ) was investigated. Climbazole is an imidazole-based antifungal compound commonly used as an anti-dandruff and antimicrobial preservative in personal care products such as shower gels, toothpaste, hair conditioners, hair tonics, and shampoos (20). The toxicity of CBZ toward various organisms, including algae, crustaceans, fish, and plants, has been investigated (21); however, its potential cytotoxic effects on cancer cells have not been thoroughly studied. There is no study showing the anticancer effects of climbazole in the studies conducted so far.

In summary, the A549 lung cancer, MDA-MB-231 breast cancer, MKN28 gastric cancer, and HT-29 colorectal cancer cell lines are indispensable *in vitro* models for understanding cancer biology and developing novel anticancer agents. The use of normal control cell lines such as HEK293 in cytotoxicity assessments plays a critical role in determining the selective anticancer potential of tested compounds. Previous studies have shown that many natural and synthetic agents exhibit strong cytotoxicity in cancer cells but not in normal cells, suggesting their potential as anticancer agents (15, 18). These findings further emphasize the importance of selective toxicity in cancer treatment and the crucial role of cell culture models in developing new therapeutic agents.

MATERIAL and METHOD

Chemicals and Instruments

Analytical-grade chemicals from Sigma-Aldrich, Merck, and TCI were used as received. Reaction monitoring was performed via TLC under UV light (CAMAG). 1H NMR spectrum was recorded at 100 MHz in CDCl3 using a Varian spectrometer (Palo Alto, USA).

Synthesis of 1-(4-chlorophenoxy)-1-(1H-imidazol-1-yl)-3,3-dimethylbutan-2-ol

The compound was synthesized according to the previously reported procedure (20-22). Briefly, CBZ (1.00 g, 3.416 mmol) and sodium borohydride ($NaBH_4$) (0.388 g, 10.247 mmol) were dissolved in a mixture of tetrahydrofuran (20 mL) and water (20 mL) in a 100 mL round-bottom flask. The reaction mixture was stirred magnetically at room temperature for 24 h, and the progress of the reaction was monitored by thin-layer chromatography (TLC). After completion, the reaction mixture was extracted with ethyl acetate (2 × 30 mL), and the combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by recrystallization from ethyl acetate/hexane to afford 1-(4-chlorophenoxy)-1-(1H-imidazol-1-yl)-3,3-dimethylbutan-2-ol (CBZ-alcohol).

The reduction reaction is illustrated in Figure 1, consistent with the previously described transformation of CBZ to CBZ-alcohol (22).

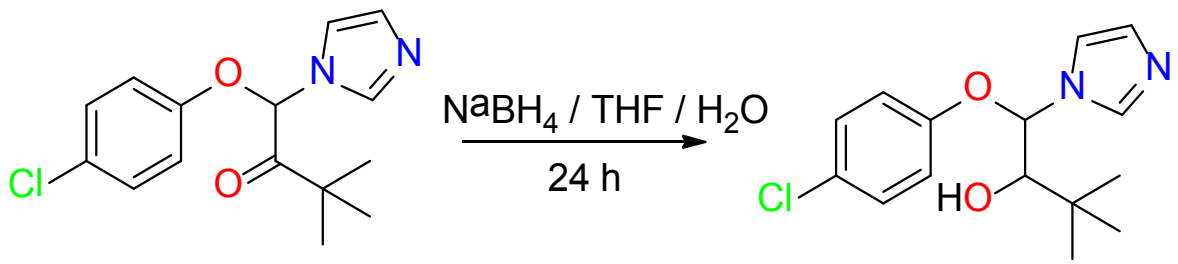

Figure 1. The schematic representation of the reduction of climbazole into climbazole-alcohol using sodium borohydride as the reducing agent (22)

Preparation of Climbazole-Alcohol Dilutions

From the compound (molecular weight 294.78 g/mol), 603.7 mg was weighed and dissolved in 10 mL of DMSO to prepare a 204.8 mM stock solution. This stock was diluted 1/100 with culture medium to obtain an intermediate concentration of 2048 µM. Serial dilutions with culture medium were then performed to prepare final concentrations of 1024, 512, 256, 128, 64, and 32 µM. These dilutions were used for cell treatment applications.

Cell Culture and Anticancer Activity Assay

Five different human cell lines were used in this study: human colorectal adenocarcinoma (HT-29, ATTC code; HTB-38), human gastric cancer (MKN28), human lung adenocarcinoma (A549/ CCL-185), human breast cancer (MDA-MB-231, ATTC code; HTB-26), and normal human embryonic kidney (HEK293, ATTC code; CRL-1573) as the control. All cell lines were obtained from the American Type Culture Collection (ATCC).

HEK293 and MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, high glucose, Gibco, USA), while A549, HT-29, and MKN28 cells were cultured in RPMI-1640 (Gibco, USA). All media were supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA). Cells were maintained in a humidified incubator at 37°C with 5% CO_2.

When cell confluence reached approximately

85-90%, the cells were detached using trypsin-EDTA (Sigma-Aldrich, USA), washed twice with PBS, and seeded into 96-well plates at a density of 1×10^4 cells per well. After 24 hours of incubation, the culture medium was replaced with 200 µL of different concentrations of Climbazole-alcohol solution (in triplicates), while control wells received only culture medium. Plates were then incubated for 72 hours.

MTT Cell Viability Assay

After incubation, the media were removed, and each well received 5 µL of 5 mg/mL MTT solution and 45 µL of culture medium (final volume 50 µL). Plates were incubated for 4 hours at 37°C. After incubation, 100 µL of 99.9% DMSO was added to dissolve the formazan crystals, and the plates were further incubated for 1 hour. Absorbance was measured at 570 nm using a Multiskan Sky Microplate Spectrophotometer (Thermo Fisher Scientific).

Cell viability (%) was calculated according to the following formula:

"Cell viability (%)"="Sample OD" /"Control OD" ×100

Determination of IC_{50} Values and Statistical Analysis

The IC_{50} value, representing the concentration required to inhibit 50% of cell viability, was determined using GraphPad Prism software. Statistical significance among viability percentages at the same concentrations was evaluated using one-way ANOVA followed by Tukey's post hoc test. A p-value of <0.05 was considered statistically significant.

RESULTS

Chemical Results

The compound 1-(4-chlorophenoxy)-1-(1H-imidazol-1-yl)-3,3-dimethylbutan-2-ol (CBZ-alcohol) was obtained by reduction of CBZ. The progress of the reaction was monitored by TLC, and the resulting product was purified by column chromatography. The physical and spectral data of the synthesized compound were found to be in good agreement with those previously reported in the literature (20-22).

Cell Viabilitiy Results

The anticancer activity of Climbazole-alcohol was evaluated against human colorectal (HT-29), gastric (MKN28), lung (A549), and breast (MDA-MB-231) cancer cell lines, along with the normal human embryonic kidney cell line (HEK293). According to MTT assay results, the compound caused a mild reduction in cell viability in a concentration-dependent manner but did not exhibit a marked cytotoxic effect overall.

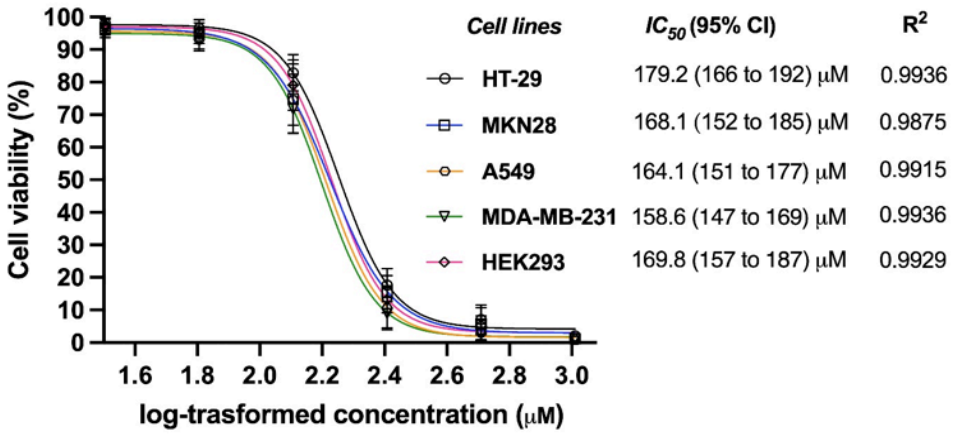

Figure 2. Dose-response curves of Climbazole-alcohol on HT-29, MKN28, A549, MDA-MB-231, and HEK293 cell lines. Cell viability (%) is plotted against logarithmically transformed extract concentrations (µg/mL). IC_{50} values, corresponding 95% confidence intervals (CI), and R^2 coefficients are indicated for each cell line

The high curve-fitting coefficients ($R^2 \geq 0.987$) confirmed the experimental reliability of the dose-response curves. The calculated IC_{50} values were 179.2 µM for HT-29, 168.1 µM for MKN28, 164.1 µM for A549, 158.6 µM for MDA-MB-231, and 169.8 µM for HEK293. These values were similar across all tested cell lines, with no statistically significant difference between the control (HEK293) and cancer cell lines ($p > 0.05$). This finding indicates that Climbazole-alcohol did not exhibit selective anticancer activity under the conditions of this study and produced comparable effects in all tested cell lines.

DISCUSSION

In this study, the in vitro cytotoxicity of Climbazole-alcohol compound was evaluated on four different human cancer cell lines (HT-29, MKN28, A549, MDA-MB-231) and the normal control cell line HEK293. The IC_{50} values obtained (HT-29: 179.2 µM; MKN28: 168.1 µM; A549: 164.1 µM; MDA-MB-231: 158.6 µM; HEK293: 169.8 µM) were within very similar ranges regardless of cell type and did not show any significant difference between cancer cell lines and normal cell lines ($p > 0.05$). This finding suggests that the compound does not exhibit selective anticancer activity, but on the contrary, has similar levels of activity in both cancer and normal cells.

The imidazole ring forms the structural basis of many biologically active molecules and has been described as a frequently used scaffold in the development of anticancer agents (23). In one study, average IC_{50} values of compounds containing an imidazole nucleus were obtained at low micromolar levels (e.g., 2.4 µM) in the NCI60 cell line panel (23). Such data suggests that imidazole-containing compounds can achieve high efficacy.

In contrast, the Climbazole-alcohol derivative used in our study exhibited relatively low cytotoxicity, with IC_{50} values in the range of 150-180 µM. This suggests that the compound requires structural modification or does not possess optimal intrinsic activity in target cells Another study reported that imidazole-chalcone derivatives had IC_{50} values in the range of 7-63 µM in the A549 cell line (24). This comparison shows that the activity of our compound is weak compared to imidazole derivatives that have shown activity at low micromolar levels in the literature.

The anticancer activities of imidazole derivatives have generally been shown to occur through apoptosis induction, cell cycle arrest (especially in the G2/M phase), and inhibition of signaling pathways (25). In this context, it may be thought that the Climbazole-alcohol structure cannot reach the relevant targets or its intracellular concentration is not sufficient. Furthermore, pharmacokinetic parameters such as lipophilicity/diffusion properties of the compound, permeation through the cell membrane, and metabolic stability may also contribute to the low efficacy.

There are several possible reasons for the low cytotoxicity of Climbazole-alcohol. Firstly, although the high lipophilicity of the compound allows it to easily cross the cell membrane, its low water solubility may have limited its effective distribution in the cell culture medium and thus its ability to reach intracellular targets. Second, the hydroxyl group in the compound's chemical structure may have weakened its pharmacophoric activity compared to the original Climbazole structure(26). Furthermore, although Climbazole-alcohol has been described as a potent antifungal agent, it appears unlikely to affect signaling pathways such as STAT3, BCL-2, or p53, key regulators of cancer cell proliferation. Some imidazole derivatives have previously been reported to induce apoptosis by inhibiting these signaling pathways (27, 28). However, MTT results indicate that Climbazole-alcohol does not exhibit such a mechanism of action.

The findings of the study demonstrate that Climbazole-alcohol does not induce selective toxicity and exhibits similar efficacy across all cell types. This suggests that the compound has a low anticancer potential. However, the imidazole ring in the Climbazole skeleton suggests that more potent anticancer derivatives could be obtained

through future structural modifications. It is anticipated that Climbazole analogs, particularly those containing halogenated or aromatic rings, may increase lipophilicity, facilitate permeation through cell membranes, and enhance interactions with target proteins (29). Furthermore, the use of Climbazole-alcohol in different combinations, such as with DNA synthesis inhibitors or oxidative stress inducers, may produce a synergistic effect (30). These approaches could be evaluated to enhance the weak anticancer activity of the compound alone.

In conclusion, this study demonstrates that the Climbazole-alcohol compound does not produce significant cytotoxic effects on various human cancer cell lines. IC_{50} values, which are close to each other and range from 158 to 179 µM, indicate that the compound does not exhibit selective activity against cancer cells, thus limiting its potential as a single anticancer agent. However, these results do not imply that Climbazole derivatives are completely ineffective; on the contrary, they suggest that pharmacological efficacy can be enhanced through structural optimization and combination strategies. Given that the imidazole backbone has been shown to have high potential in the literature (e.g., derivatives exhibiting nanomolar IC_{50}) (27), the Climbazole structure can be considered a lead compound and more effective derivatives can be developed through optimization studies.Future studies should aim to investigate in detail the effects of different chemical modifications of Climbazole derivatives on cell cycle regulatory proteins, oxidative stress responses, and apoptosis markers (e.g., BAX, BCL-2, CASPASE-3).

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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