RESEARCH ARTICLE

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Anti-Angiogenic and Anti-Proliferative Activities of 5-Bromo-N-(2,5-Dioxopyrrolidin-1-Yl)-1H-Indole-2-Carboxamide

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Abstract

Background: Angiogenesis has long been a key focus for drug designers aiming to develop therapies targeting diseases associated with this physiological process. A newly synthesized compound from the University of Baghdad was evaluated for its ability to inhibit blood vessel growth. The objective of this study was to investigate the anti-angiogenic activity of 5-bromo-N-(2,5-dioxopyrrolidin-1-yl)-1H-indole-2-carboxamide using an ex vivo rat aorta model. **Methods:** An anti-angiogenesis assay was employed to assess the dose-response relationship and to determine the concentration that inhibits 50% of blood vessel growth (IC_{50}). The anti-proliferative effect on endothelial cells was assessed using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay. Additionally, the free radical scavenging activity of the compound was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The anti-proliferative activity against the A549 lung cancer cell line was also investigated. **Results:** The compound demonstrated significant anti-angiogenic activity with an IC_{50} of 15.4 µg/mL. The IC_{50} on the HUVEC cell line was 5.6 µg/mL. Its free radical scavenging activity was measured at 99.6 µg/mL. Furthermore, the compound significantly inhibited the proliferation of the A549 lung cancer cell line, with an IC_{50} of 14.4 µg/mL. **Conclusion:** The findings suggest that 5-bromo-N-(2,5-dioxopyrrolidin-1-yl)-1H-indole-2-carboxamide possesses notable anti-angiogenic activity and a significant anti-proliferative effect on HUVEC cells, potentially linked to its strong free radical scavenging capacity. Moreover, it effectively inhibited the proliferation of lung cancer cells.

Keywords: Angiogenesis- cell line- free radical scavenging activity- MTT assay

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Introduction

Angiogenesis is a fundamental process in the human body that orchestrates the growth of new blood vessels from pre-existing ones. At its core, angiogenesis involves a delicate balance of pro-angiogenic and anti-angiogenic signals [1]. While the body tightly regulates this process under normal circumstances, aberrant angiogenesis can lead to significant health issues. Understanding the intricacies of angiogenesis offers promising avenues for therapeutic intervention in diseases such as cancer, cardiovascular disorders, and chronic inflammatory conditions. Several efforts in this area of research have led to the discovery of many synthetic compounds with potential pharmacological targets for anti-angiogenesis.

The process of angiogenesis unfolds through a series of well-coordinated steps. Initially, it is triggered by various stimuli, such as hypoxia, inflammation, or growth factors like vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) [2]. These stimuli prompt endothelial cells, which line the interior surface of blood vessels, to activate and proliferate, laying the foundation for new vessel formation. In cancer, for example, tumors exploit angiogenesis to ensure a sufficient blood supply for their growth and metastasis. By secreting pro-angiogenic factors, cancer cells induce the formation of new blood vessels, facilitating their nutrient uptake and dissemination to distant sites. Targeting angiogenesis has thus emerged as a promising strategy for cancer therapy, with anti-angiogenic drugs designed to inhibit tumor vascularization and impede disease progression [3]. A new synthetic compound has been synthesized in the University of Baghdad's Department of Pharmaceutical Chemistry. The aim of this article is to provide insight into the role of

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angiogenesis in various diseases, focusing on the signaling pathways of angiogenesis activators and inhibitors in both physiological and pathological conditions.

Materials and Methods

Chemistry

The 5-bromo-N-(2,5-dioxopyrrolidin-1-yl)-1H-indole-2-carboxamide derivative was synthesized at the Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad. The compound was fully investigated and characterized using various spectroscopic techniques [4-8].

Experimental animal

Male albino rats with an average weight of 250 g were used in this experiment. The animals were obtained from the Animal House of Al-Nahrain University, College of Pharmacy. The animals were provided with all the required facilities and had free access to food and tap water. The experiments were approved by the Animal Ethics Committee, College of Pharmacy, Al-Nahrain University [9-12].

Cell culture

Human umbilical vein endothelial cells (HUVECs) and the A549 lung cancer cell line were purchased from the American Type Culture Collection (ATCC, USA). Cells were cultured in F-12K Medium (for HUVECs) and RPMI-1640 medium (for A549 cells), both of which were supplemented to create complete growth media according to ATCC instructions. Cells were maintained at 37 °C and 5% CO_2. For the anti-proliferation assay (MTT), HUVEC and A549 cells were treated for 48 hours with serial dilutions of 4-2-(5-bromo-1H-indol-2-carbonyl)-N-(4-methoxyphenyl) hydrazine-1-carbothioamide [13].

Angiogenesis

The angiogenesis assay was conducted according to Hayder and colleagues [14]. The animals were anesthetized with diethyl ether, and the aorta was sectioned into rings. The rings were cultured in complete medium, and 10 µL of thrombin was added to each well. The compound was serially diluted with M199 medium, and the 48-well plates were incubated at 37 °C and 5% CO_2 for five days. Blood vessel outgrowth was observed on day five. The percentage of blood vessel inhibition was calculated using the following formula:

Inhibition (%) = 1 - [(A) / (A_0)] × 100,

where:

A: Average distance of blood vessel outgrowth in the sample (µm)

A_0: Average distance of blood vessel growth in the control.

Free Radical Scavenging Activity (DPPH Assay)

The antioxidant activity of the test compound was evaluated by measuring its free radical scavenging activity using the DPPH assay. The compound was prepared in methanol at six different concentrations (1000, 500, 250, 125, 62.5, and 31.5 µg/mL). A 200 µL DPPH solution was added to 100 µL of each compound concentration. After 30 minutes of incubation in the dark, absorbance was measured at 517 nm using a plate reader. The tests were performed in triplicate. Free radical scavenging activity was calculated using the formula:

F = [(A) / A0] × 100%

where:

A_0: Absorbance of the control

A: Absorbance of the sample [15]

Results

Serial concentrations of the synthetic compound exhibited varying degrees of blood vessel inhibition. At concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL, blood vessel inhibition was observed at 99%, 87%, 57%, 33%, 26%, and 18%, respectively. The IC_{50} value was calculated using the straight-line equation derived from the dose-response curve shown in Figure 1 below, yielding an IC_{50} of 15.4 µg/mL.

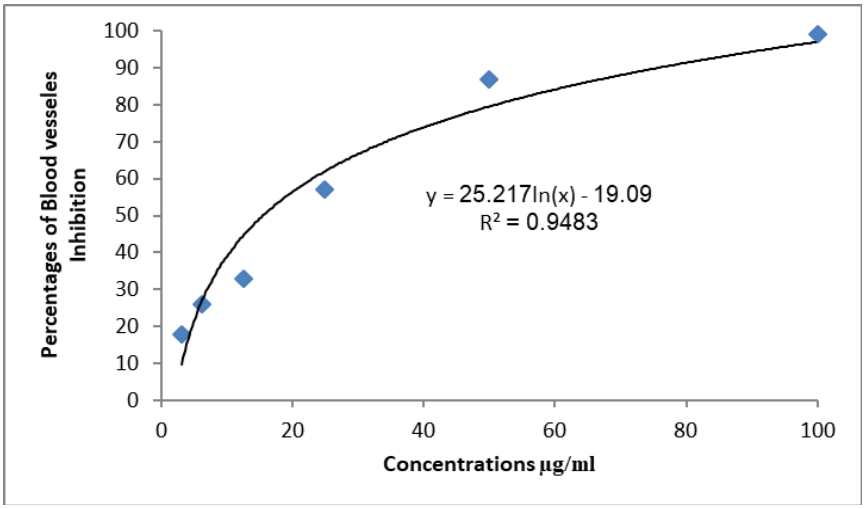

Figure 1. Dose-Response Curve of 5-bromo-N-(2,5-dioxopyrrolidin-1-yl)-1H-indole-2-carboxamide against New Blood Vessel Growth.

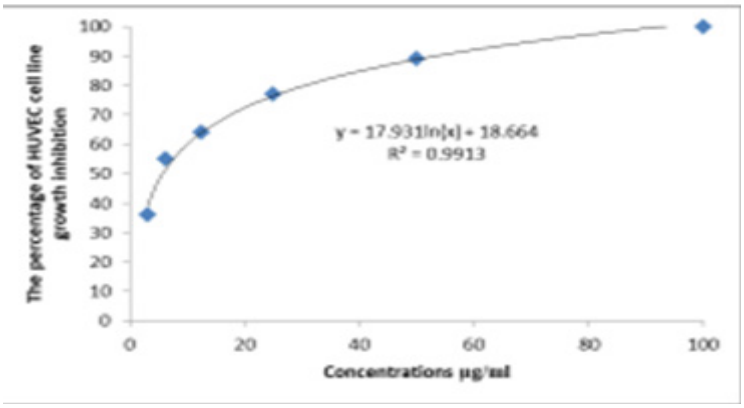

Figure 2. Dose-Response Curve of the Anti-Proliferative Activity of the Synthetic Compound against the HUVEC Cell Line.

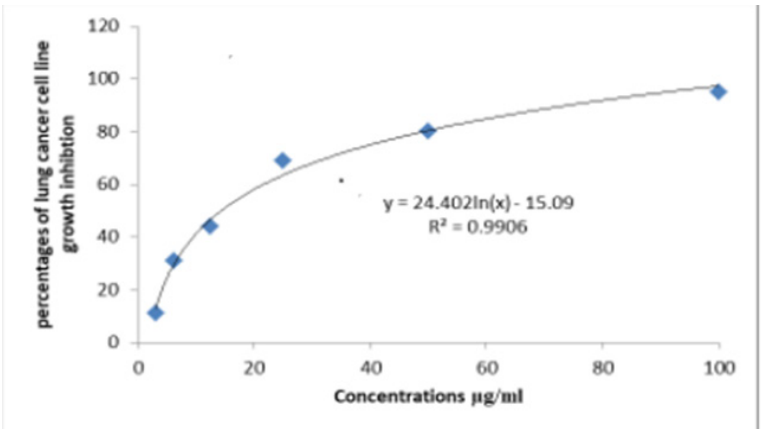

Figure 3. Dose-Response Curve of the Anti-Proliferative Activity of the Synthetic Compound against the A549 Lung Cancer Cell Line.

Anti-Proliferative Activity of 5-Bromo-N-(2,5-Dioxopyrrolidin-1-yl)-1H-Indole-2-Carboxamide Against HUVEC and A549 Lung Cancer Cell Lines

Figures 2 and 3 illustrate the anti-proliferative activity of the synthetic compound against the HUVEC cell line (Figure 2) and the A549 lung cancer cell line (Figure 3), as assessed by the MTT assay. The indole derivative exhibited a dose-dependent anti-proliferative effect on both cell types. For HUVECs, concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL inhibited cell proliferation by 100%, 89%, 77%, 64%, 55%, and 36%, respectively. The IC_{50} value for HUVECs was 5.6 µg/mL. In the case of A549 lung cancer cells, the compound inhibited cell proliferation by 95%, 80%, 69%, 44%, 31%, and 11% at the same concentrations, with an IC_{50} value of 14.4 µg/mL.

Free Radical Scavenging Activity of 4-2-(5-Bromo-1H-Indol-2-Carbonyl)-N-(4-Methoxyphenyl) Hydrazine-1-Carbothioamide

Figure 4 shows the free radical scavenging

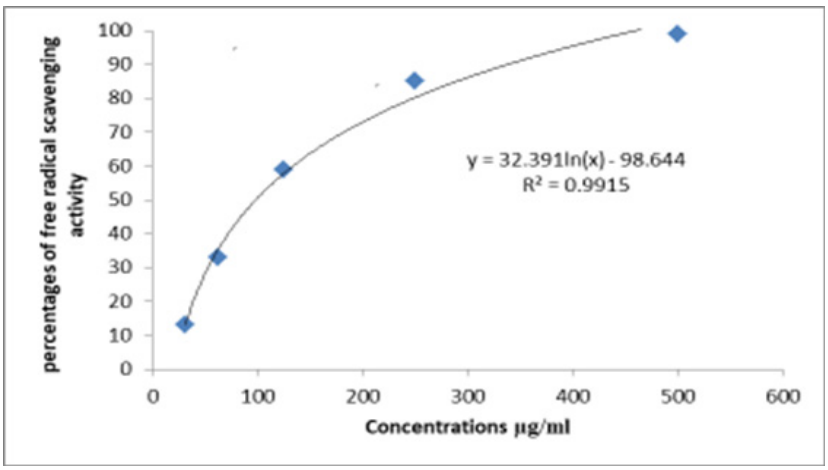

Figure 4. Free Radical Scavenging Activity of the Synthetic Compound.

activity of 4-2-(5-bromo-1H-indol-2-carbonyl)-N-(4-methoxyphenyl) hydrazine-1-carbothioamide. At concentrations of 500, 250, 125, 62.5, and 31.25 µg/mL, the compound exhibited scavenging activity of 99%, 85%, 59%, 33%, and 13%, respectively. The IC_{50} was calculated to be 99.6 µg/mL.

Discussion

This study presents promising preclinical data for the synthetic compound, which was synthesized and characterized by scientists at the University of Baghdad [9-12]. The encouraging results served as a catalyst for further investigation into the compound's mechanism of action as an anti-angiogenic agent. Additionally, it was crucial to evaluate the compound's anticancer activity, as angiogenesis plays a significant role in cancer progression [16]. The compound demonstrated a marked ability to inhibit blood vessel sprouting, prompting an investigation into its potential to inhibit endothelial cell proliferation. The data revealed a strong effect, with the compound significantly inhibiting the proliferation of the HUVEC cell line, which may help elucidate its pharmacological activity. Given that the compound is an indole derivative, it is noteworthy that previous studies have shown indole compounds to significantly inhibit VEGF [17]. Due to the compound's pronounced effect on blood vessel perturbation and its anti-proliferative activity against HUVEC cells, it was essential to further assess its ability to inhibit cancer cell proliferation in vitro. The compound exhibited significant anti-proliferative activity against the lung cancer cell line in a dose-dependent manner. However, the IC_{50} was not within the cytotoxic range, indicating that the compound primarily exerts an anti-angiogenic effect, with cytotoxicity observed only at higher concentrations [14]. This finding is consistent with the study by Hayder and colleagues in 2014 [18]. The investigation of free radical scavenging activity was crucial to understanding the potential mechanism of action of the compound. The data presented in this study revealed a significant dose-dependent effect in scavenging free radicals, suggesting that the compound may exert its effects through antioxidant properties [19]. Oxidative stress, a key factor in the pathogenesis of many diseases, can lead to angiogenesis. Antioxidants have been shown to reduce oxidative stress and may significantly influence neovascularization [20]. Moreover, the impact of antioxidants on angiogenesis and the factors regulating this process is well-documented. Most synthetic antioxidants exhibit an inhibitory effect on neovascularization, and antioxidant compounds have demonstrated their ability to inhibit angiogenesis and inflammation, as well as reduce endothelial cell growth stimulated by FGF-2 and VEGF [18, 19]. The anti-angiogenic properties of antioxidant compounds are thought to be mediated by their influence on VEGF signaling, as well as the tyrosine phosphorylation of vascular endothelial cadherin and β-catenin [21].

Author Contribution Statement

All authors contributed equally in this study.

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Conflict of interest

None.

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