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In vitro Activity of Novel Cannabinoids Derived from Tetrahydrocannabinolic Acid on Various Human Tumor Cell Lines

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ABSTRACT

The in vitro study of tetrahydrocannabinolic acid (THCA) derivatives ALAM027 and ALAM108 was carried out on the following human tumor cells: T47D (breast, ductal carcinoma), PC-3 (prostate, adenocarcinoma), HT-29 (colorectal carcinoma), Caco-2 (colon, adenocarcinoma), A549 (lung, carcinoma), U87MG (human glioblastoma) and U266B1 (multiple myeloma).

The ALAM027 compound showed good growth inhibition in all cell lines tested with the exception of U87MG cells. The ALAM108 compound also suppressed the growth of U87MG cells but had little effect on T47D tumor cells.

In vitro studies of THCA derivatives ALAM027 and ALAM108 showed antitumor activity in all cell lines tested. The difference in the activity of these compounds in relation to the T47D and U87MG tumor cells may be indicative of different functional mechanisms.

Keywords:
THCA
Cannabinoids
T47D
PC-3
HT-29
Caco-2
A549
U87MG
U266B

1. Introduction

Tetrahydrocannabinolic acid (THCA) is the main component of Cannabis sativa. However, in contrast to its derivative THC, the biological properties of THCA have been studied to a much lesser extent, particularly because it is difficult to isolate and because of its high sensitivity to heat and UV radiation. A convenient and inexpensive method has recently been described to isolate this acid using ion-exchange resins, opening up the way to industrial scale production and making THCA a suitable starting product for drug synthesis [12]. This advance has recently facilitated the synthesis of two THCA derivatives, ALAM027 and ALAM108, which exhibit good anti-tumor activity in PANC-1 and AsPC-1 cell lines [3].

Since natural cannabinoids such as THC and CBD are known to have broad-spectrum anti-tumor activity against many types of tumors [4-6], it is of interest to investigate a potential effect of ALAM027 and ALAM108 on various types of cancer cells. According to World Health Organization data the most widespread types of cancers are breast, lung, colon, intestine, pancreatic, prostate tumors and blood diseases such as multiple myeloma. Brain tumors such as gliomas are also potentially interesting, particularly because of their aggressive and highly invasive properties [7]. To facilitate comparisons between previously reported activities of natural cannabinoids and the
ALAM027 and ALAM108 compounds, the current study examines the effects of these compounds on the following human cancer cell lines: T47D (breast, ductal carcinoma), PC-3 (prostate, adenocarcinoma), HT-29 (colorectal carcinoma), Caco-2 (colon, adenocarcinoma), A549 (lung, carcinoma), U87MG (human glioblastoma) and U266B1 (multiple myeloma).

All these tumor cell lines express significant levels of CB1 and CB2 receptors in their cell membrane and this amount increases with cell proliferation. Thus, several articles have been devoted to studying the effects of THC and CBD on tumor cell line T47D which is often used in breast cancer research [8-11].

The PC-3 cell line is also often used in prostate cancer research. Studies examining the effects of cannabinoids on PC-3 cells have predominantly focused on elucidating tumor growth suppression mechanisms [12,13].

The HT-29 cell line is a colorectal tumor line which is often used as an epithelial cell model to study new drug candidates because of its ability to differentiate. Cannabinoids have a significant effect on HT-29 cells as they induce cell death through apoptosis and inhibit proliferation [14]. The role of cannabinoid receptors in these processes has been studied by examining effects of agonists such as THC and CBD on cancer cells in the presence and absence of CB1 and CB2 antagonists [15].

Similarly to HT-29 cells, natural cannabinoids also suppress colorectal adenocarcinoma Caco-2 cell growth by inducing apoptosis and inhibiting cell proliferation which is mediated through CB1 receptor binding [16,17].

A549 is one of the most widely studied lung tumor cell line, which is often used as a testing ground for new drugs, such as natural and synthetic cannabinoids because the main determining factor of the anticancer effect of these cannabinoids on A549 cells is their ability to block the CB1 receptor, which is overexpressed in non-small cell lung tumors [18-20].

Brain glioblastoma occupies an important place among studies of the anticancer activity of cannabinoids. A significant number of research studies have focused on the effects of THC and CBD ligands on U87 MG, in particular because of their rather substantial in vitro and in vivo activities [21-23]. It is interesting to note that, in contrast to SF126 glioblastoma cells, in the case of U87MG cells THC does not exhibit a pleiotropic effect at 1 µM concentration and below [23]. Multiple myeloma is one of the most serious hematological diseases and is characterized by drug resistance. Cannabinoids are among the most promising candidates for the treatment of this disease. Recent research [24] indicates that the IC50 values for CBD and THC in U266 cells are 19.8 µM and 39.5 µM respectively, and their combined use leads to the synergistic increase of cytotoxic effects when compared to their individual activities.

The present activity study of the ALAM027 and ALAM108 compounds in these selected tumor cell lines will not only allow to assess their anticancer activity but also, to a certain extent, could be used to understand their putative functional mechanisms.

2. Materials and Methods

Synthesis data and spectral characteristics of THCA derivatives ALAM027 and ALAM108 have been described previously [8]. The in vitro study was performed on T47D, PC-3, HT-29, Caco-2, A549, U87MG and U266B1 cells obtained from the Chempartner (China) collection using the CellTiter Glo Viability Assay. Cells were seeded in 96-well plates in a volume of 100 µl per well, according to the planned plate layout and a predefined seeding density. Plates were incubated in a CO2 incubator overnight. The compound stock solution was diluted with DMSO to a 200 × final concentration, and serial 3-fold dilutions prepared from a 2-mM solution (final concentration range: 0.5-10000 nM for 10 doses). An internal staurosporine control was included on each plate. A volume of 0.5 µl of diluted compound was added to appropriate wells according to the plate layout. The plates were incubated at 5% CO2, 37°C for 72 hours. After this incubation, CellTiter-Glo reagents were prepared and added, and the plates read in an Envision plate reader. Inhibition and IC50 for each of the compounds were calculated with the XLFit curve fitting software (n=2, Z Factor, SW).

3. Results and Discussions

The structures of THCA and its derivatives ALAM 027
Figure 1. Growth inhibition curves of T47D, PC-3, HT-29, Caco-2, A549, U87MG and U266B1 cells at a wide range of THCA, ALAM027, and ALAM108 concentrations.

For the T47D cell line, the ALAM027 compound shows good growth inhibition with an IC50 value comparable to THC (6.7 µM) and CBD (5 µM) \cite{8}. The ALAM108 compound is less active, though its ability to inhibit cell proliferation significantly exceeds that of THCA.

The inhibition values of both cannabinoids on PC-3 prostate tumor cells are very similar (Table 1) but the ALAM027 compound yields a pleiotropic effect at 1 µM concentration following the growth inhibition.

The comparison of both cannabinoid activities in
HT-29 cells shows that these cells are more sensitive to ALAM108 than to ALAM027 while IC50 values of both compounds are much higher than those of CBD (23-30 µM) and THC (30 µM) \[14\]. However, in the case of Caco-2 cells the IC50 values differ slightly and are comparable to those obtained in PC-3 cells.

The effect of ALAM027 and ALAM108 on A549 cells is practically the same both in terms of the degree of inhibition and IC50. When compared to THC (27.2 µM) and CBD (37.1µM) \[19\] this advantage becomes clearly evident.

Regarding the U87MG cell line, compound ALAM027 shows a low-level activity comparable to THC. Cannabinoid ALAM108 effectively inhibits cell survival with an IC50 of 3.37 µM that is on par with the activity of THC (IC50 1.2-14 µM) and CBD (IC50 1.5-9.7 µM) in this cell line \[21\]. One of the possible reasons may be the ability of ALAM108 to pass through the blood-brain barrier due to its greater hydrophobicity (LogP 5.81) compared to ALAM027 (LogP 4.38). Perhaps this assumption is very relative, but currently available cannabinoid anticancer activity data on U87MG cells only relates to THC, CBD and some synthetic cannabinoids like WIN55,212-2 \[18\-23\].

The comparison of both cannabinoid activity against U266B1 cells shows the advantage of ALAM108 as for other cell lines.

**Table 1.** IC50 and inhibition values (10 µM) of THCA and its derivatives on T47D, A549, PC-3, HT-29, Caco-2, U87 MG, and U266B1 tumor cells.

<table>
<thead>
<tr>
<th>Cancer cell lines</th>
<th>THCA</th>
<th>ALAM027</th>
<th>ALAM108</th>
<th>Parameters of screening assay*</th>
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<tr>
<td></td>
<td></td>
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<td>µM</td>
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<td>U266B1</td>
<td>8.68</td>
<td>&gt;10</td>
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</table>

* [25]

**4. Conclusions**

Our current in vitro study of THCA derivatives ALAM027 and ALAM108 showed their antitumor activity in all the tumor cell types examined. The difference in the activity of these compounds in relation to the T47D and U87MG tumor cells may be indicative of different functional mechanisms.

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**Author Disclosure Statement**

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**References**


