Hormetic effects of *in vitro* anticancer activity of cannabinoid acid derivatives in the HT-29 colon cancer cell line

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Abstract:

Aim: The *in vitro* anticancer activity of THCA and CBDA cannabinolic acid derivatives **1-4** was investigated in the HT-29 colon cancer cell line.

Methods: The effects of compounds ALAM138 (**1**), ALAM027 (**2**), ALAM108 (**3**) and ALAM146 (**4**) on HT-29 cell growth were measured using the XTT assay (72 hours).

Results: Compounds **1,2,4** showed a significant hormetic effect in the 0.5-2 uM concentration range. The very slight hormetic trend observed for 3 shows the advantage of a THCA cannabinoid structure over CBDA.

Conclusion: The *in vitro* anticancer activity of several THCA and CBDA derivatives in the HT-29 colon cancer cell line indicates the presence of a hormetic proliferative effect that is dependent on the cannabinolic acid and substituent structures.

Keywords: Cannabinoids, Hormesis, THCA, CBDA

Introduction

The anticancer activity of cannabinoids and their derivatives is one of the important directions in the search for new marijuana-based drugs [1-3]. This is particularly pertinent for the most common malignant diseases of the gastrointestinal tract and accessory organs, namely cancers of the small intestine, colon and pancreas. The main focus in the search for potential anticancer candidates among products derived from cannabis has so far turned to derivatives such as THC, CBD and CBG on a smaller scale. Their precursors, the cannabinolic acids and their derivatives, have been studied to a much lesser extent, even though these compounds have clear advantages in many of their characteristics such as biocompatibility, solubility in biological fluids, and the absence of psychoactive properties [4].

The *in vitro* activity of cannabinolic acid derivatives ALAM027 and ALAM108 in a variety of cancer cell lines [5] was found to induce a slight increase in cell proliferation at low test substance concentrations.

Although this deviates from the classical model of a concentration-dependent reduction in cell growth with increasing anticancer drug concentrations, the proliferative effect may be explained by experimental error or by a potential hormetic effect as has indeed been established for many of the currently deployed anticancer drugs [6, 8]. The present work, therefore focusses on investigating the anticancer activity of a number of cannabinolic acid derivatives in the HT-29 colon cancer cell line using the XTT assay. The HT-29 cell line was chosen because it is accessible and has been previously shown to have a propensity for hormetic responses to anticancer drugs [6].

Materials and methods

Compounds ALAM138 (**1**), ALAM027 (**2**), ALAM108 (**3**) and ALAM146 (**4**) were obtained from natural THCA and CBDA as previously described [7]. The HT-29 cell line (ATCC® HTB-38™) was obtained from the collection of the Pharma Seed company (Israel). For the *in vitro* assay, HT-29 cells were seeded in 96-well plates at a density of 7500 cells/well in culture medium. Cells were allowed to attach for 16-24 hours at 37°C, 5% CO_2. Culture medium was then discarded, and fresh assay medium added and supplemented with either an increasing concentration of compounds **1-4** or 1% DMSO as a negative control. Cells were then incubated for another 72±2 hours at 37°C, 5% CO_2. At the end of incubation period, 100 µL fresh culture medium was added to the cells along with 50 µL XTT reagent [2,3-Bis (2-methoxy-4-nitro-5-sulfophenyl0-2H-tetrasolium-5-capboxanilide inner salt]. The optical density (OD) was measured in a plate reader. All assays were performed in triplicate. Vehicle treated cells reached an OD at the 450 nm wavelength ranging from 0.5-1.5 (Average ± SEM).

Results

Compounds **1** and **2** are derived from THCA and differ in their amide structures (Figure 1). This is in contrast to compounds **3** and **4** that have the same hydrazide substituent but are obtained from different cannabinolic acids (Figure 1).

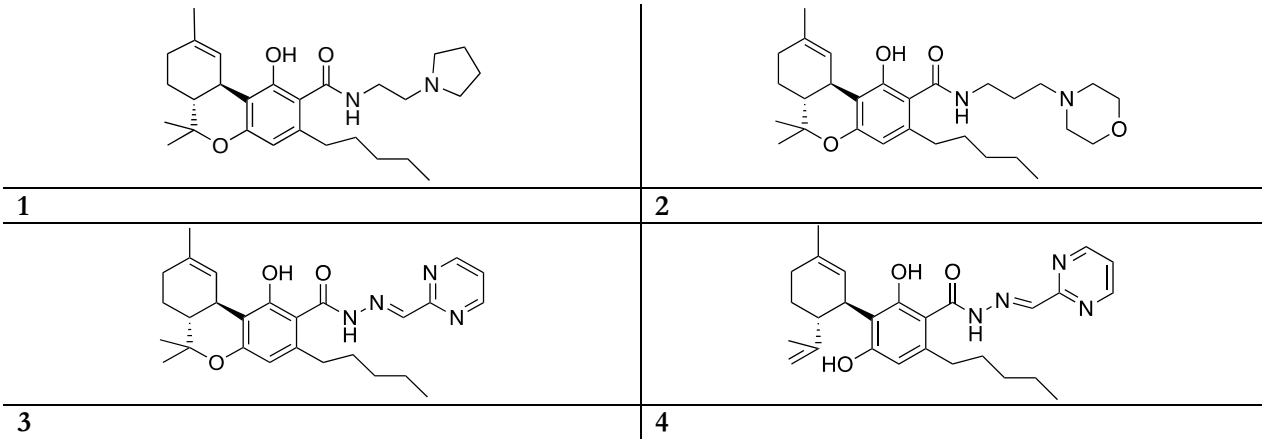
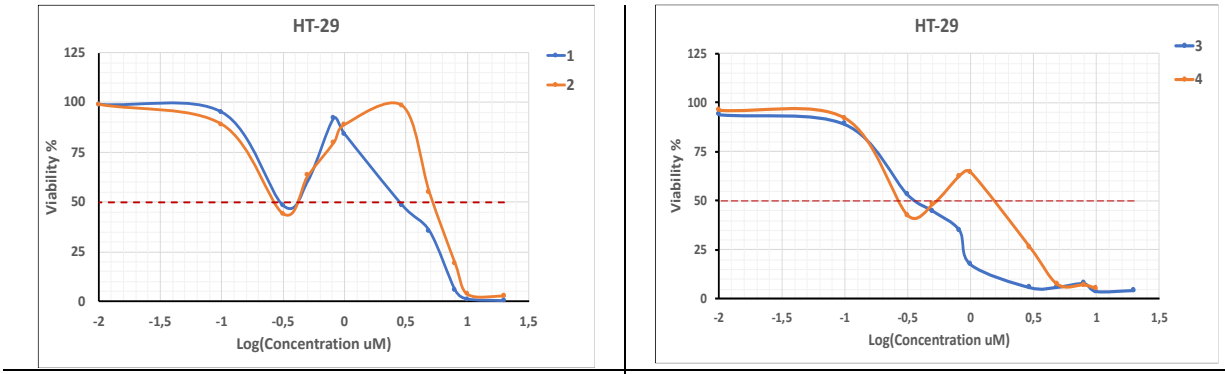


Figure 1. The structures of THCA derivatives 1-3 and CBDA derivative 4.

The HT-29 cancer cell line viability curves with increasing concentrations of cannabinoid compounds **1, 2** (A) and **3, 4** (B) follow a hormetic response characterized by a growth inhibition and stimulation phase at lower compound concentrations, followed by inhibition of growth at increasing compound concentrations (Figure 2).



A B

Figure 2. The dose-response curves of compounds 1, 2 (A) and 3, 4 (B) activity on HT-29 cell line viability (3 repeats, 72 hours, Average ± SEM).

For compounds **1,2,4** IC50 values can be considered along with other curve parameters namely Mh and h/c IC50 which are obtained by considering the 50% value as the base line. Since compound **3**, only shows a very slight rise in the curve around the 1 uM concentration, it is unclear whether this is due to hormesis or an experimental deviation, and we are omitting the Mh and h/c IC50 data points for this compound. The remaining dose-response curve parameters are shown in Table 1.

Table I. Dose-response curve parameters: hIC50 - IC50 of hormetic reaction; cIC50 - IC50 of cytotoxicity; MS - maximum stimulation. The base line was considered to correspond to 50% viability.

Compounds	hIC50 uM	cIC50 uM	MS %
1	0.301	3.09	44
2	0.263	4.89	50
3		0.331	
4	0.319	1.62	15

Discussion

E.J. Calabrese defined hormesis as "an adaptive response to low levels of stress or damage resulting in improved fitness for some physiological systems for a finite period" [6]. For cancer cells this means activation of cell growth at specific drug concentrations and suppression of growth at increased drug concentrations as has been shown in *in vitro* studies of a number of widely used drugs in the A549 lung cancer cell line (Figure 3). Figure 3A depicts a number of theoretical dose-response models of anticancer drugs. The hormetic response is described by the following variables: Mh the maximal value of the hormetic reaction, hED50 the value of the hormetic reaction, cED50 the value of cytotoxicity, RT the reduction threshold (the drug concentration on y = Baseline) and MS the maximum stimulation (the highest stimulation rate). All of these parameters allow to quantify the contribution of the hormetic reaction. Figure 3B shows the dose-response curves of some widely used anticancer drugs [8].

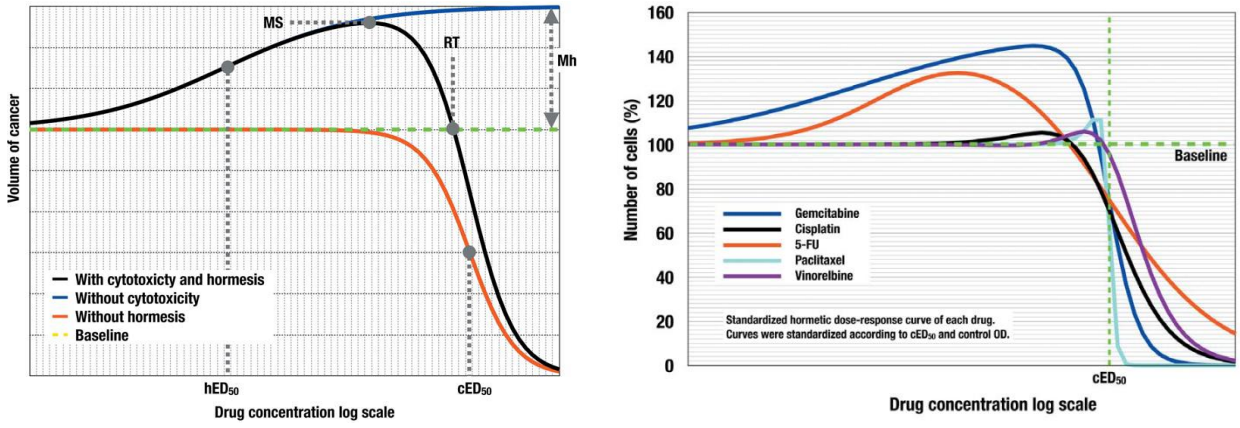


Figure 3. A: Theoretical dose-response models for anticancer agents with hormesis; B: The dose-response curves of five commonly used anticancer agents. Curves were standardized according to cED50 and control OD [8].

Hormetic effects have also been identified in *in vitro* studies of some of the cannabinoids. The article by Fowler [9] for example describes results from a 66-hour incubation of four different lines of pancreatic cancer cells with Δ9-THC. Δ9-THC concentrations of 3 and 4 uM completely inhibited cell growth in all cell lines tested. However, a concentration of 1 uM Δ9-THC on PANC-1 and 0.5 uM on Capan2 cells significantly increased cell viability, but this was not observed in BxPc3 and MiaPaCa2 cells.

As shown in Table 1, the propensity of colon cancer HT-29 to hormetic effects in the presence of anticancer drugs [6] allowed to reveal the influence of cannabinoid structures **1-4** on the balance between their real anticancer activity and activation of the hormetic response. Paradoxically the main contribution to the final cIC50 values for **1,2,4** is the hormetic effect, whilst neither the structure of the cannabinoid moiety nor the nature of the carbonyl substituent has a large effect on the hIC50 value. The very slight hormetic reaction of compound **3** shows the advantage of a THCA cannabinoid structure over CBDA and makes it the most interesting for further study.

This current *in vitro* study investigating the anticancer activity of several THCA and CBDA derivatives in the HT-29 colon cancer cell line shows the presence of a hormetic proliferative effect that is depended on cannabinolic acid and substituent structures.

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Author Disclosure Statement

The author has no conflicts of interest, and no competing financial interests exist.

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