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ARTICLE

Investigating the *in vitro* Antitumor Structure-activity Relationship of a Range of Cannabinolic Acid Derivatives

Alexander Aizikovich*

AL & AM Pharmachem Ltd. Carmel St. 5, Rehovot, Israel

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ABSTRACT

Aim: To investigate the *in vitro* structure-activity relationship (SAR) of a range of tetrahydrocannabinolic (THCA) and cannabidiolic (CBDA) derivatives using the PANC-1 tumor cell line (pancreas, ductal carcinoma). **Materials and methods**: The *in vitro* effects of a range of THCA and CBDA derivatives with different carbonyl group substituents were tested on the PANC-1 cells cell line using the CellTiter Glo Viability Assay (72 hours) and the XTT assay (48 hours). **Results:** A study of a series of THCA and CBDA derivatives containing different functional groups at the carbonyl nitrogen atom demonstrated that THCA amides have better inhibitory activity, on the PANC-1 tumor cell line, than CBDA derivatives. **Conclusions:** THCA derivatives have better inhibitory activity than CBDA analogs with the same substituents. It is noteworthy that even a slight change in the structure of the substituent of the amide or hydrazone moiety of the molecule has a dramatic effect on the activity of these compounds.

1. Introduction

The interest in cannabinoids, as potential anticancer drugs, has grown considerably in recent times, as evidenced by the large number of reviews and articles devoted to these compounds [1-5]. This is particularly true for compounds such as CBD and THC, as they are relatively readily available and stable; however, their limited synthetic potential and low bioavailability mean that the structure of cannabinoids needs to be modified to obtain potentially novel drugs [6,7]. This is in contrast to their precursors, the cannabinolic acids, which present significantly greater opportunities due to the presence of a carboxyl group in the o-position of the phenolic hydroxyl. Intensive research on cannabinolic acids has not only provided supportive evidence for their anti-inflammatory and anticancer activity, but, more importantly, the absence of any psychoactive properties. The more widespread use has however been hampered by difficulties related to isolating these acids with a sufficient degree of purity and

*Corresponding Author:
Alexander Aizikovich,
AL & AM Pharmachem Ltd. Carmel St. 5, Rehovot, Israel;
Email: alexaizik53@gmail.com

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their tendency to decarboxylate. A relatively simple and inexpensive isolation method has recently facilitated the synthesis of a number of carboxyl group derivatives and some of these compounds have shown *in vitro* and *in vivo* anticancer activity in several types of tumor cells [8,9].

This current work aims to investigate the structure-activity relationship of THCA and CBDA derivatives with different carbonyl group substituents on a range of cancer cell types.

2. Materials and Methods

The synthesis and spectral characteristics of ALAM027 **4** and ALAM108 **12** have been described elsewhere [8]. The *in vitro* function of both compounds was tested on cell lines, from the Chempartner (China) collection, using the CellTiter Glo Viability Assay (Table 1). Briefly, cells were incubated with the individual compounds for 72 hours at 5% CO_2 and 37 °C, as previously described [8]. The half maximal inhibitory concentration (IC50) of each individual compound as well as their inhibitory effect at 10 uM was determined with the XLFit curve fitting software (n = 3, Z factor > 0.8; SW >10 [11]).

The inhibitory activity of compounds **1-12** was also tested *in vitro* on the PANC-1 (ATCC® CRL-1469™) cell line from the Pharma Seed company (Israel). PANC-1 cells were plated 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. The culture medium was then discarded and fresh assay medium added to the cells and supplemented with increasing concentrations for each individual compound. DMSO at 1% was used as a negative control. Cells were subsequently incubated for another 48±2 hours at 37 °C, 5% CO_2. At the end of incubation period, 100 µL of 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]. Optical density (OD) was measured in a plate reader once vehicle treated cells reached an OD 450 nm range of 0.5-1.5 (Average ± SEM).

3. Results

The two THCA derivatives, ALAM027 (**7**) and ALAM108 (**12**) (Figure 1) have been previously shown to inhibit the growth of a variety of tumor cell lines *in vitro* as well as suppress the *in vivo* outgrowth of human PANC-1 in the pancreatic tumor xenograft model [10]. The activity of these substances on 14 cancer cell lines derived from a variety of organs such as the brain, lungs, esophagus, liver, pancreas, intestines, prostate, and blood cancer such as myeloma [8] are shown below.

Table 1. ALAM027 (**7**) and ALAM108 (**12**) *in vitro* IC50 and inhibition (10 uM) results from the CellTiter Glo assay (72 hours).

Cancer cells	**7**		**12**		**STS***	
	Inhibition %	IC_{50} uM	Inhibition %	IC_{50} uM	Inhibition %	IC_{50} uM
T47D breast	97.90	5.52	47.20	>10	83.46	0.22
U251 brain	68.11	8.91	-	-	95.87	0.01
U-87 MG brain	19.84	>10	73.80	3.37	94.92	0.08
A549 lung	77.08	5.59	70.01	5.53	93.20	0.01
TE-6 esophagus	67.27	7.36	-	-	100	0.01
Caco-2 colon	60.81	8.87	67.16	6.56	91.56	0.01
HT-29 colon	86.21	6.27	88.13	1.99	99.03	0.02
OPM-2 myeloma	76.40	6.72	-	-	100	0.01
U266B1 myeloma	58.33	8.20	73.05	4.52	95.81	0.01
SK-HEP-1 liver	78.20	5.41	-	-	100	0.01
PC-3 prostate	61.13	9.94	63.61	7.45	99.83	0.02
PANC-1 pancreas	92.19	5.46	85.71	1.88	99.82	0.04
AsPC-1 pancreas	64.62	7.78	67.69	4.46	88.40	0.02
MiaPaCa-2 pancreas	61.41	8.12	90.65	1.76	98.05	0.03

*Staurosporine (standard)

These encouraging results warranted further investigation of several of these THCA and CBDA derivatives in the PANC-1 pancreatic cancer cell line model using the XTT assay (48 hours) to determine their anticancer activity.

The structures of compounds **1-12** are detailed below (Figure 1).

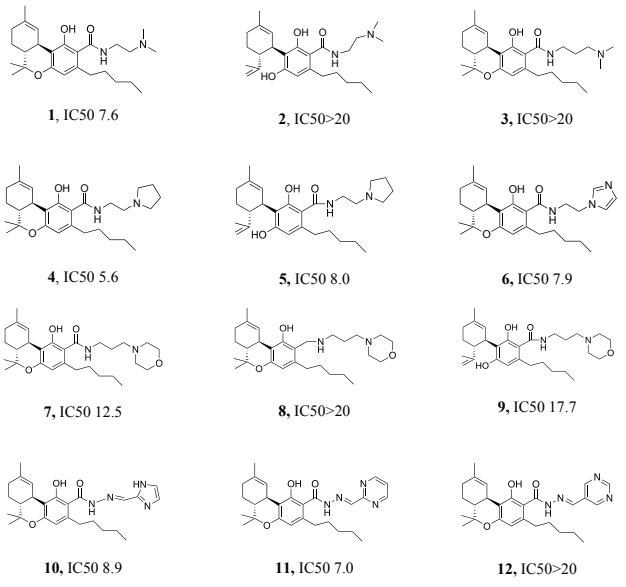

Figure 1. Structures of THCA and CBDA derivatives **(1-12)** and their IC50 (uM) on PANC-1 tumor cells (Cells inhibition was measured with the XTT assay, after 48-hour exposure to each individual compounds).

4. Discussion

Tumor cells have been shown to express significant levels of CB1 and CB2 receptors on their cell membranes, with the receptor concentration increasing as the tumor grows. Consequently, substances that bind to these receptors are able to block cancer cell growth. This activity has been successfully demonstrated for the natural cannabinoids THC and CBD as well as several synthetic compounds [4,5]. The significant differences between the chemical structures of these compounds do not however allow to consider the contribution of specific parts of the molecule to biological activity. Since the cannabinolic acid derivatives investigated in the current study retain the integrity of their key cannabinoid fragment, their activity can be dissected using a classical structure-activity approach. All compounds contain two or more nitrogen atoms and can be subdivided into two groups: amides and hydrazides. The presence of a tertiary amino group in the amide fragment increases polarity and may contribute to increasing the bioavailability of cannabinolic acid derivatives. Furthermore, hydrazides are known for their high level of inhibitory activity in many types of cancer cells [8].

As shown in Figure 1, THCA derivatives are more inhibitory than CBDA analogs with the same substituents. Even a slight change in the structure of the substituent both in the amide and hydrazone moieties of the molecule appears to have a dramatic effect on the activity of these compounds. Replacement of the 2-pyrimidyl ring with the 4-isomer resulted in a loss of antitumor activity for compound **11** compared to **12.** Introduction of a 2-imidazolyl ring resulted in a slight decrease in the inhibitory activity of compound **10** which highlights the importance of the presence of nitrogen atoms in specific positions of the heterocyclic fragment. Reducing the carbonyl group of compound **4** to methylene led to the loss of inhibitory activity in compound **5.** Compound **3** also lost anti-tumor activity due to an increase in alkyl chain length when compared to compound **1**. THCA derivative **4** with its ethane-2-N-pirrolidine substituent had the highest inhibitory activity of all compounds tested on the PANC-1 cell line.

5. Conclusions

This study of a series of THCA and CBDA derivatives with different functional groups at the carbonyl nitrogen atom position showed (i) that THCA amides **1, 4, 7** had greater inhibitory activity than CBDA derivatives **5** and **9** in PANC-1 tumor cell line and, (ii) that even minor variations in substituent structure of the hydrazide moiety of compounds **10, 12** resulted in dramatic changes in antitumor activity.

The present work demonstrates that a wide range of THCA and CBDA derivatives are capable of inhibiting cancer cell proliferation and therefore offer the prospects of cost-effective drugs with potent antitumor activity across a range of tumor cell types.

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Conflicts of Interest

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

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