A Review of Endocannabinoid System and its Pharmacological Role as a Drug Target for Cancer Therapies.

By
MD Salehin Khan
19146013

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of pharmacy (Hons.)

School of Pharmacy
BRAC University
February 2023

Declaration

It is hereby declared that

1. The project submitted is my/our own original work while completing degree at Brac University.
2. The project does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The project does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

MD Salehin Khan
19146013

Approval

The thesis titled “Endocannabinoid system and its pharmacological role as a drug target for cancer therapies” submitted by MD Salehin Khan (19146013) of Summer, 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

Supervised By:

_____

Tanisha Momtaz
Lecturer
School of Pharmacy
BRAC University

Approved By:

Program Director:

_____

Professor Dr. Hasina Yasmin
Program Director and Assistant Dean
School of Pharmacy
BRAC University

Dean:

_____

Professor Dr. Eva Rahman Kabir
Dean
School of Pharmacy
BRAC University

Ethical Statement

This study work does not involve any kind of trials with lab animals nor involves human trials.

Abstract

Cancer is a genetic disorder that causes uncontrolled cell proliferation and the cells are unable to maintain apoptosis which leads the cells to cross their natural boundaries. Treatment of this diseases requires a complex system of medicines for this purpose endocannabinoid system serves as a potential target for effective medicine development. Endocannabinoid system is homeostasis system present in mammalians. It comprises of endogenous and exogenous ligands, membrane bound receptors and degrading enzymes. The ligands of this system can be an effective molecule for drug development. Cannabinoid molecule can cause apoptosis in cancer cell through three different pathways they include JNK–and p38 MAPK activation pathway, ceramide generating pathway and autophagy pathway. By modulating these pathways cannabinoid can be effective against various types of cancer cells. These molecules also have anti-inflammatory and analgesic properties and evidence shows that these properties can be useful in palliative care treatment with less side effects.

Keywords: Proliferation, Cannabinoid, Endocannabinoid system, Apoptosis, JNK and p38 MAPK pathway, Ceramide, Autophagy.

Dedication

This work is dedicated to my parents, friends and family.

Acknowledgement

Firstly, I would like to thank my supervisor Tanisha Momtaz madam, Lecturer, School of Pharmacy, Brac University for helping me with every problem that I faced during paper writing, ma'am was very helpful and was available when I need her help, she was very cooperative and helpful throughout whole time.

I am also grateful to Professor Dr. Eva Rahman Kabir, our honorable Professor and Dean of the School of Pharmacy at Brac University.

Lastly, I would like to give a special thanks to Namara Mariam Chowdhury madam for giving us a wonderful basics of anti-cancer medicines and also to all my faculty members for making my university life so wonderful.

Table of contents

List of tables

List of Figures

List of Acronyms

ECS	Endocannabinoid system
GPCR	G-protein coupled receptor
CB2	Cannabinoid Receptor 1
CB2	Cannabinoid Receptor 2
AC	Adenylyl cyclase
cAMP	Cyclic adenosine monophosphate
2-AG	2-arachidonoylglycerol
THC	Tetrahydrocannabinol
ATF-4	Activating transcription factor 4
PKA	Protein kinase A
TRIB3	Tribbles Pseudokinase 3
MAPK	Mitogen activated protein kinase
JNK	c-Jun N-terminal kinase
EMT	Endocannabinoid membrane transporter
NAT	N-acyltransferase

Chapter 1

Introduction

1.1 Brief history

Among various physiological systems in human body the endocannabinoid system (ECS) is the most diverse one. Endocannabinoid system is a very recently discovered less than 30 years ago. It is a biological homeostasis system in human and as well as animals this system comprises of membrane receptors their endogenous ligands and their degrading enzymes these degrading enzymes are also involved in maintaining ligands life cycle (Silver, 2019). This diverse system was named after discovery source cannabis plant and this *cannabins sativa plant* contains a group of chemical compounds known as cannabinoid, a single cannabis plant throughout its life cycle can produce more than 500 types of cannabinoids (Laezza et al., 2020a). In 1988, a prominent and globally known cannabinoid scientist Vincenzo Di Marzo, discovered the relationship between endocannabinoid and human physiology, this relationship includes mood swing, sleep, memory, perception to pain and also in maintaining balance in the body however Dr. Raphael Mechoulam is said to be the godfather of cannabis research as he was the first person to discover and isolated tetrahydrocannabinol (THC) and cannabidiol (CBD) in 1964 (Behl et al., 2022). In Weizmann Institute, Israel, Raphael Mechoulam for his research work somehow succeeded to collect 5 kg of cannabis that was seized by police and identified psychoactive compound by testing it on monkeys and after his work a novel homeostasis system, the endocannabinoid system came into lime light (Crocq, 2020). ECS has many potentials to offer in modern medicinal world, this mysterious system is unraveling itself with works of various renowned scientists who dedicated their life to find out the potentials of this system so that modern world can have the pharmacological benefits endocannabinoid system.

1.2 ECS: Endocannabinoid system and its receptors

ECS has very vast and diversified function in biological system it has role in gastrointestinal system, cardiovascular system, nervous system. It also modulates immune system, autonomic nervous system and involved in micro circulation at regions outside the brain, depending upon receptor activation and deactivation ECS can be helpful in treating various pathological condition. Generation of psychoactive effects like euphoria by endocannabinoid system can be consider to be the reason of cannabin’s substances abuse. Endocannabinoid system is

composed of two types of receptors, first one is central cannabinoid receptor 1 (CB1) and the second one is peripheral cannabinoid receptor 2 (CB2) (Murillo-Rodríguez, 2008) these two receptors are G-protein coupled receptor (GPCR) (Leo & Abood, 2021)(Albertin et al., 2016). CB1 receptor is most abundant GPCR in brain and mostly found the peripheral and central neuronal cells terminus, glial cell , reproduction mainly testes and other glands in human , CB1 receptor modulates bodily functions like motor and cognitive behavior ,emotional responses and maintains homeostasis in brain and CB2 receptor is associated with immune cells, it is expressed in many lymphoid organs, B lymphocytes, polymorphonuclear neutrophils and monocytes, T-lymphocytes has the least expression of CB2 receptor (Behl et al., 2022). CB1 receptor protein consist of 7 transdermal domains and in an adult human brain CB1 receptors are distributed in some special regions that includes thalamus, hypothalamus, cortex, hippocampus, limbic system, basal ganglia and also spinal cord (Glass et al., 1997). Upon activation it acts through G_i alpha subunit (Axelrod1 & Felder1, 1998) and calcium channel blockade and potassium channel activation occur resulting decrease adenylyl cyclase (AC) activity and reduced cAMP production but this receptor activates PLC and this phenomenon describes the behavioral effects upon CB1 activation (Murillo-Rodríguez, 2008). CB2 receptors are mainly distributed in the myocardium and human coronary, this receptor is also seen endothelial and smooth muscle cells, CB2 receptors found extensively in the brain and in immune cells (Albertin et al., 2016). Principally in immune cells CB2 receptors are located in leucocytes in spleen and tonsils (Pertwee, 2001). In immune system activation of CB2 receptor of B and T cells results in inhibition of adenylyl cyclase which reduces immune response (Condie et al., 1996). CB2 receptors are also involved neural and non-neural cells survival, their differentiation and proliferation. There is many evidence showing that CB2 receptor have to potential to induce apoptosis and also have the ability to inhibit tumor growth in host mice (Sviženská et al., 2008).

1.3 Endogenous and exogenous ligands

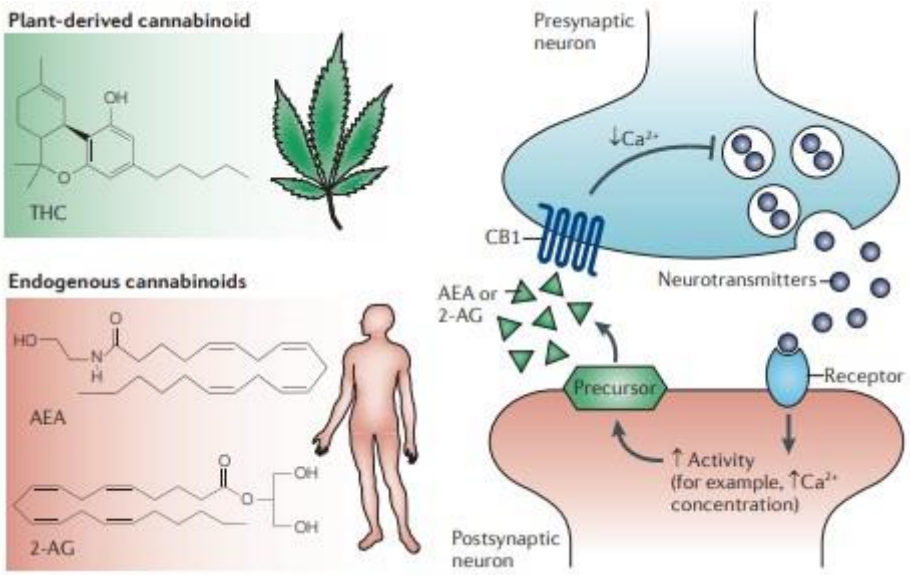


Figure 1 | Endocannabinoid and exocannabinoid (Guzmán, 2003).

The above figure shows activation path ways of specific cannabinoid receptor by both phyto cannabinoids and endocannabinoids. Δ9 -tetrahydrocannabinol (THC) is a phyto cannabinoid, it activates cannabinoid receptors. Endocannabinoid receptors are normally activated by endocannabinoids a neuromodulating molecules, two of these prominent neuromodulators includes anandamide N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG). When neurotransmitter binds to receptor it activates postsynaptic neurons to make neuromodulator endocannabinoid, after releasing these precursors molecules they increase the concentration of calcium ions in cytoplasm (Katona & Freund, 2008). Endocannabinoids sometimes binds to presynaptic CB1 cannabinoid receptors and inhibits Ca2+ influx into the cell which blocks release of neurotransmitter this features allows to tune some biological features like as memory, movement, appetite and pain (Guzmán, 2003).

Cannabinoids works as a ligand group for cannabinoid receptors. Cannabinoids are compounds containing of C_{21} terpenophenolic group (Mechoulam & Gaoni, n.d.) cannabinoids can be classified endocannabinoids and phytocannabinoids. Both endocannabinoids and phytocannabinoids are ligands for cannabinoid receptors, the basic difference between them is endocannabinoids are neurotransmitters synthesized in mammalians so it can be said to be endogenous ligands on the other hand phytocannabinoids are plat-based compounds synthesized in marijuana plants and said to be exogenous ligands. Endocannabinoids are present in brain and periphery of humans, these compounds are produced from cultured

neurons, microglia and astrocytes and macrophages (Scotter et al., 2010). Till now five endogenous ligands or endocannabinoids are identifies they include anandamide which is arachidonoyl ethanolamide stands for AEA, 2-AG stands for 2-arachidonoyl glycerol, virodhamine for which chemical name is O-arachidonoyl ethanolamine and NADA which stands for N-arachidonyldopamine. Both biosynthesis and degradation of these cannabinoids molecules occurs through hydrolytic enzyme for example in biosynthesis of 2-arachidonoylglycerol (2-AG) the enzymes involved includes PLC standing for phospholipases C and DAGL standing for diacylglycerol lipases these class of enzymes show sn-1-selective reaction , NAT with a chemical name of N-acyltransferase these enzymes used in acetylation reaction and lastly NAPE-PLD with a chemical name of N-acylphosphatidyl-ethanolamine-specific phospholipase on the other hand for degradation of arachidonoyl ethanolamide AEA the hydrolytic enzyme involved is FAAH standing for fatty acid amide hydrolase. Finally release and reuptake of endocannabinoids occurs in pre and post synapses of neurons and transportation happens through endocannabinoid membrane transporter (EMT) (di Marzo et al., 2004). The pathway through which 2-AG is synthesized is shown below (Ueda et al., 2011).

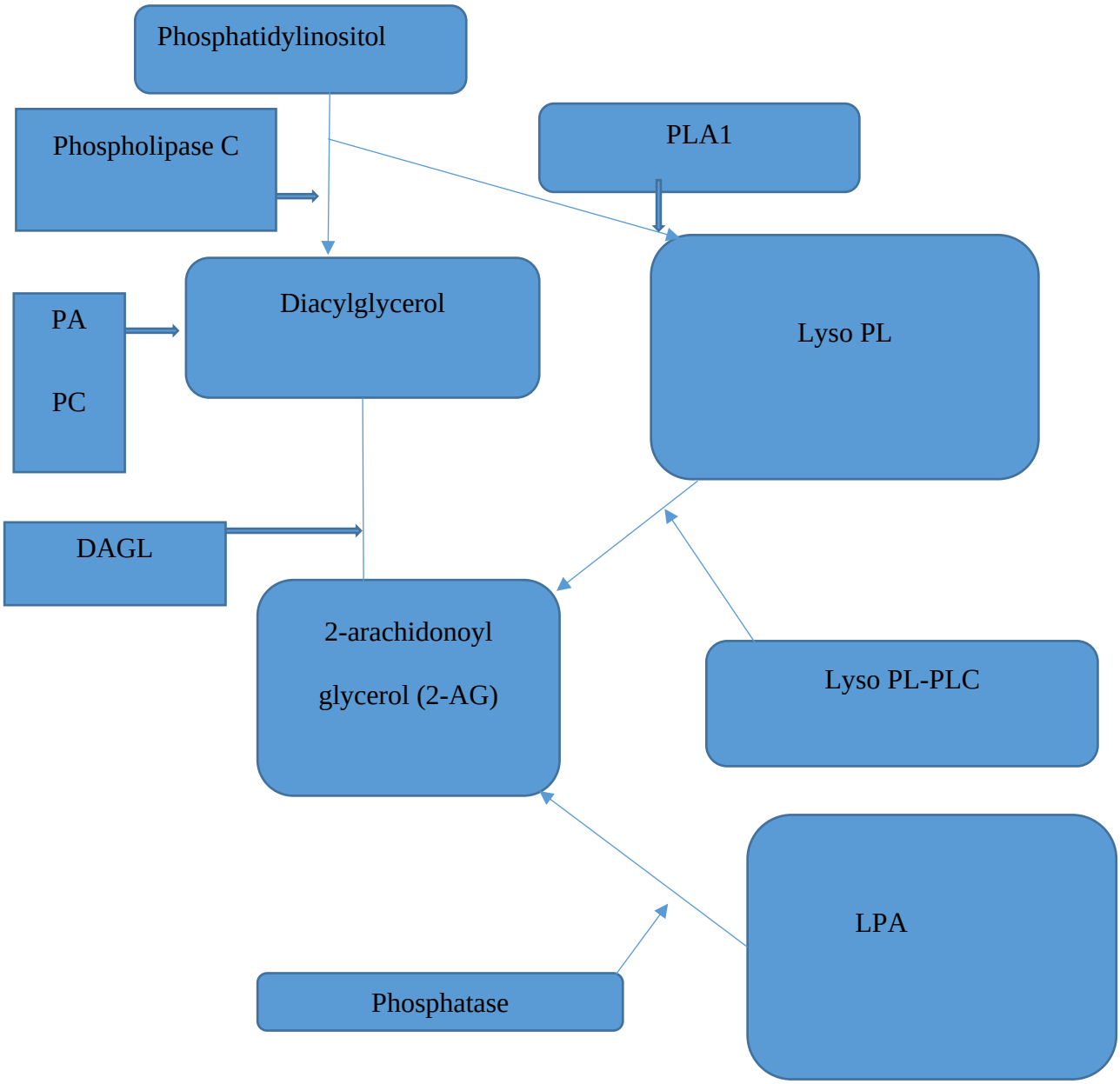
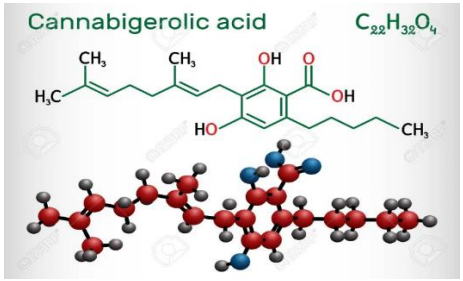


Figure 2: Biosynthetic pathway for 2 AG

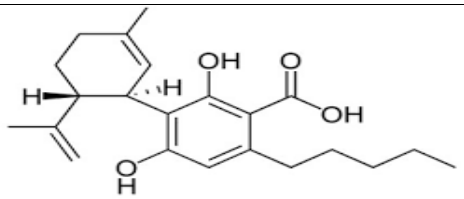
Till now 66 cannabinoids are identified and they are classified into 10 subclasses (Elsohly, n.d). Among 10 sub classes only a few of the compounds have pharmacologically significant properties, these classes are categorized based on the basic structures of the molecules that have common features. Some of the significant sub classes are mentioned in the table below, their structural similarities are mentioned below.

Table 01 Cannabichromene classes (Elsohly, n.d).

Compounds under examination	Structural information	Pharmacological activity
$C_{22}H_{32}O_4$ Cannabigerolic acid (CBCA)	 In the above structure if we consider COOH a R1 position and C5H11 as R2 position we can discuss other compounds of this class based on this structure.	
Cannabichromene (CBC)	If we replace R1 and R2 with hydrogen and C5H11 in basic structure we will get CBC.	CBC has Analgesic and anti-inflammatory properties. It also shows antifungal and antibacterial properties.
Cannabichromevarinic acid (CBCVA)	By replacing R1 and R2 with COOH and C3H7 we can get CBCVA	

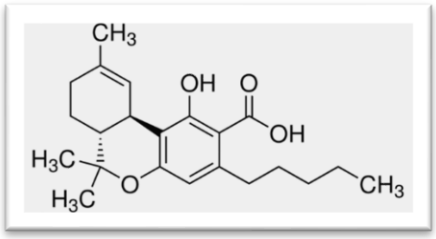
Cannabichromevarin (CBCV)	By replacing R1 and R2 with hydrogen and C3H7 we will get CBCV from basic structure.	

Table 02 Cannabidiol class (Elsohly, n.d).

Compounds under examination	Structural information	Pharmacological activity
Cannabidiolic acid (CBDA)	 CBDA (Cannabidiolic Acid) On this basic structure if we consider COOH as R1, C5H11 as R2 and, H3 we can describe the below compounds.	Antibacterial properties.
Cannabidiol (CBD)	By replacing R1, R2and R3 with hydrogen C5H11 and hydrogen we will get CBD	Pharmacological properties of CBD include analgesic, anxiolytic and anti-inflammatory properties. Also have anti-oxidant, antispasmodic and antipsychotic effects
Cannabidiol monomethylether (CBDM)	R1=H, R2=C5H11, R3=CH3	

CBD-C_4, Cannabidiol -C_4	If we replace R1 with hydrogen and, R2 and R 3 with C4H9 and hydrogen of the basic structure we will get CBD-C_4	
CBDVA Cannabidivarinic acid	If we replace R1, R2 and R3 with COOH, C3H7 and hydrogen in the basic structure we will get CBDV.	
CBDV, Cannabidivarin	By replacing R1, R2 and R3 with hydrogen, C3H7 and hydrogen in basic structure we will get CBDV)	
CBD-C_1, Cannabidiorcol	R1=H, R2=, R3=H By replacing R1, R2 and R3 with hydrogen, CH3 and hydrogen in basic structure we will get CBD-C_1	

Table 03 Delta-9-tetrahydrocannabinol class (Elsohly, n.d).

Compounds under examination	Structural information	Pharmacological activity.
THCA-A with chemical name of Delta-9-tetrahydrocannabionlic acid A.	 If we consider COOH as R1, C5H11 as R2 and R3 as hydrogen we can describe below structure	
(THCA-B), Delta-9-tetrahydrocannabinoli acid B	By replacing R1, R2 and R3 with hydrogen C5H11 and COOH we can get THCA-B from basic structure	
(THC) Delta-9-tetrahydrocannabionl	By replacing R1, R2 and R3 with hydrogen, C5H11 and hydrogen we can get THC from basic structure.	This compound has very broad range of pharmacological properties these includes Euphoria, analgesic and anti-inflammatory properties. Other effects include antioxidant and antiemetic effects. This molecule also

		shown in vivo inhibitory effect on lung cancer (i.e., after oral administration in mice)(Guindon & Hohmann, 2011)
THCA-C_4, Delta-9-tetrahydrocannabionlic acid-C_4	By replacing R1, R2 and R3 with COOH, C4H9 and hydrogen we can get THCA-C_4 from basic structure. OR By replacing R1, R2 and R3 with hydrogen, C4H9 and COOH we can get THCA-C_4 from basic structure.	
THC-C_4 with chemical name of Delta-9-tetrahydrocannabionl-C_4	By replacing R1, R2 and R3 with COOH, C4H9 and hydrogen we can get THC-C_4 from basic structure.	
Delta-9-tetrahydrocannabivarinic acid (THCVA)	By replacing R1, R2 and R3 with COOH, C3H7 and hydrogen we can get THCVA from basic structure.	
THCV chemically known as Delta-9-tetrahydrocannabivarin.	By replacing R1, R2 and R3 with hydrogen, C3H7 and hydrogen we can get THCV from basic structure.	Works as analgesic and exerts euphoria
THCA-C_1 known as Delta-9-tetrahydrocannabiorolic acid.	By replacing R1, R2 and R3 with COOH, CH3, and hydrogen we can get THCA-$C_1$from basic structure. H OR By replacing R1, R2 and R3 with hydrogen, CH3, and COOH, we can get THCA-$C_1$from basic structure	

THC-C_1 chemically known as Delta-9-tetrahydrocannabiorol.	By replacing R1, R2 and R3 with hydrogen, CH3, and hydrogen, we can get THC-$C_1$from basic structure.	
Delta-7-cis-iso-tetrahydrocannabivarin		

Table04 Delta-8-tetrahydrocannabinol class (Elsohly, n.d).

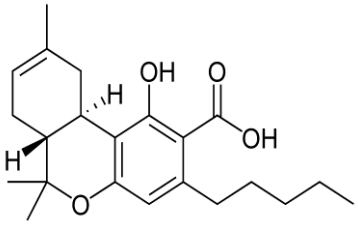
Compounds under examination	Structural information	Pharmacological activity
Delta-8-THCA chemically known as Delta-8-tetrahydrocannabinolic acid.	 If we consider COOH as R1 and C5H11 as R2 we can describe other molecules of this class	
Delta-8-THCA with chemical name of Delta-8-tetrahydrocannabinol.	By replacing R1 and R2 with hydrogen and C5H11 we can get Delta-8-THCA from basic structure.	

Table 05 Cannabicyclol class (Elsohly, n.d).

Compounds under examination	Structural information	Pharmacological activity
CBLA with chemical name of Cannabicyclolic acid		

	If we consider COOH and C5H11 as R1 and R2 we can describe other structures of this class.	
CBL chemically known as Cannabicyclol.	By replacing R1 and R2 with hydrogen and C5H11 we can get Cannabicyclol (CBL) from basic structure.	
CBLV chemically known as Cannabicyclovarin	By replacing R1 and R2 with hydrogen and C3H7 we can get CBLV from basic structure.	

Table 06 Cannabieisoin class (Elsohly, n.d).

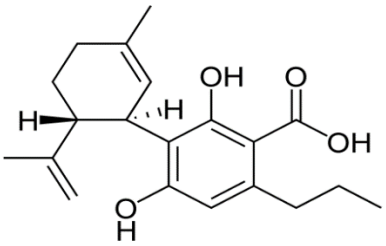
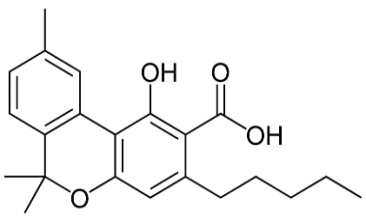
Compounds under examination	Structural information	Pharmacological activity
CBEA-A chemically known as Cannabielsoic acid A.	 If we consider COOH, C5H11 and hydrogen as R 1 ,2 and 3 we can describe other structures of this class.	
CBEA-B, Cannabielsoic acid B	By replacing R1 R2 and R3 with hydrogen, C5H11 and COOH we can get CBEA-B from basic structure.	
CBE with chemical name of Cannabielsoin.	By replacing R1 R2 and R3 with hydrogen, C5H11 and hydrogen we can get CBEA-B from basic structure.	

Table 07 Cannabinol and cannabinodiol class (Elsohly, n.d).

Compounds under examination	Structural information	Pharmacological activity
CBNA with chemical name of Cannabinolic acid A		

	If we consider hydrogen, COOH and C5H11 as R1 3 we can describe other structures of this class.	
Cannabinol (CBN)	By replacing R1 R2 and R3 with hydrogen, hydrogen and C5H11 we can get CBN from basic structure.	Have pharmacological properties including Sedation, antibacterial, anticonvulsant and reduces inflammation.
Cannabinol methylether (CBNM)	By replacing R1 R2 and R3 with CH3, hydrogen and C5H11 we can get CBNM from basic structure.	
Cannabinol-C_4 (CBN-C_4)	By replacing R1 R2 and R3 with hydrogen, hydrogen and C4H9 we can get CBN-C_4 from basic structure.	
Cannabivarin (CBV)	By replacing R1 R2 and R3 with hydrogen, hydrogen and C3H7 we can get CBV from basic structure.	
Cannabinol-C2(CBN-C2)	By replacing R1, R2 and R3 with hydrogen, hydrogen and C2H5 we can get CBN-C2 from basic structure	
Cannabiorcol (CBN-C1)	By replacing R1, R2 and R3 with hydrogen, hydrogen and CH3 we can get CBN-C2 from basic structure.	
Cannabinodiol (CBND)	R=C5H11	
Cannabinodivarin (CBVD)	R=C3H7	

Table 08 Cannabitrol class (Elsohly, n.d).

Compounds under examination	Structural information	Pharmacological activity

Cannabitriol (CBT)	 If we consider hydrogen, OH and C5H11 as R1, R2 and R3 we can easily describe other structures of this class by using this structure as a basic structure.	
10-Ethoxy-9-hydroxy-delta--9-hydroxy-delta	By replacing R1, R2 and R3 with OH, hydrogen and C5H11 we can get 10- Ethoxy-9-hydroxy-delta-9-hydroxy-delta from basic structure	
8,9-Dihydroxy-delta-6a-tetrahydrocannabinol	R1=OH, R2= H, R3=C5H11	
Cannabitriolvarin (CBTV)	By replacing R1with hydrogen, R2 with hydroxyl and R3 with C3H7 we will get CBTV	
Ethoxy-cannabitriolvarin (CBTVE)	By replacing R1 with hydrogen, R2 with OC2H5 and R3 with C3H7 we will get CBTVE from basic structure	

All the miscellaneous cannabinoid are under class 9.

1.4 Aim

Focus of this study is to provide an overview of endocannabinoid system and its endogenous and exogenous ligands and how receptor and ligands of this system can be used in concept of cancer therapy.

1.5 Objective

The objectives of these research work include an initial overview of different aspects of endocannabinoid system. Secondly it holds pharmacological properties of different cannabinoid molecules and anti-cancer properties.

Chapter 2

Research Methodology

The research work was conducted using various reliable online databases like research gate, PubMed, ScienceDirect Elsevier and open Athens account from university library was very helpful for this purpose from these sources some review article, systemic review article and some research article was gathered some chapter from books were also used. Key ideas were market and a rough outline was designed. Based on the outline different articles specific to ECS and cancer was isolated and those articles was the basic of this paper. Searching for key words on article was done through Microsoft edge. For referencing Mendeley desktop with citation setting American psychological association 7th edition with US English language was used.

Chapter 3

Cancer therapy and endocannabinoid system

Cancer is a disease that involves uncontrolled cell proliferation, cells grow beyond their natural boundaries, divide and invade surrounding tissue and triggers angiogenesis(Suryadev et al., 2017) . Generally, cancer occurs due to genetic changes, changes in gene allows cancer cell to show abnormal cell growth, depending on the genetic changes cancer cell ca be of various types, can happen on various parts and even same type of cancer may have cells with different genetic changes in different patients. Target cancer therapies refers to a special class of cancer therapies that uses specific target protein that is involved in cancer cell growth and progression. Drugs used in target cancer therapies looks for special molecular identification in cancerous cells and by binding to target they slow down cancer growth and progression. Cannabinoids can modulate several different pathways which is involved in the growth of the cell, differentiation of cells, movement and angiogenesis of cancerous cells (Laezza et al., 2020b). Cannabinoid molecules can show their anti-cancer properties by following their separate mechanism these mechanisms involve inhibition of tumor angiogenesis, invasiveness, metastasis and stimulation of autophagy which involves accumulation of ceramide and lastly modulation of the anti-tumor immune response (Vecera et al., 2020).

3.1Anticancer and anti-tumoral Effects of Cannabinoids

From late 90s a large number of evidence of experimental data suggest anti-tumor effect of cannabinoids in cell lines and mice's that were engineered genetically for lab testing (Velasco et al., 2012). Endocannabinoids including 2-AG and AEA and other synthetic cannabinoids which shows activity for cannabinoid receptors, some synthetic compounds like methanandamide, WIN 55,212-2 or HU-210 shows a higher affinity for CB1 then CB2 on the other hands compounds like JWH-133 shows higher affinity for CB2 compared to CB1 receptors these compounds play a key role in anti-tumoral activity, these findings strongly support the notion of endogenous cannabinoid system in cancer. Antitumorigenic activity can be achieved from pharmacologic stimulation of CB receptors.(Vecera et al., 2020)

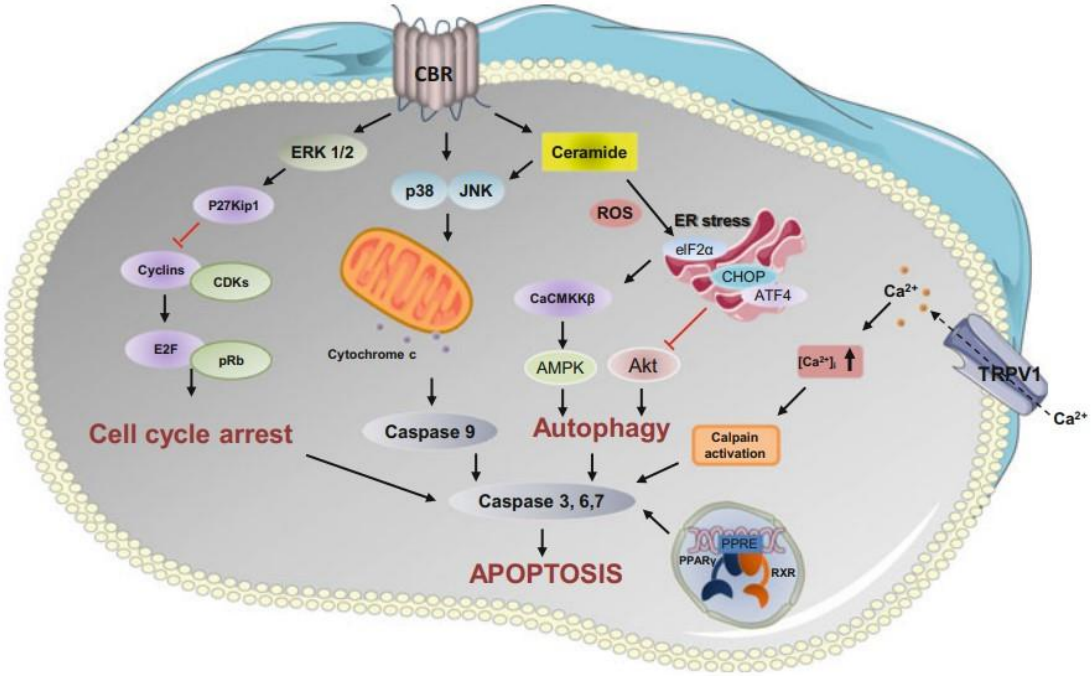


Fig 03: systemic representation of induced apoptosis through cannabinoid receptor (Fonseca et al., 2017).

There are three main signaling pathways involved in cannabinoid induced apoptosis , the first pathway involves JNK and p38 MAPK activation , after activation they stimulates mitocho ndria to release cytochrome c following this event activation of caspase-9 and -3/-6/-7 occurs which results apoptosis of cell .In the second pathway of apoptosis generation of ceramide occurs that engages first pathway or produces reactive oxygen species ROS which affects the membrane of endoplasmic reticulum (ER) and generates stress in ER . This stressing action on endoplasmic reticulum (ER) stimulates third pathway which is autophagy. Now autophagy can occur in two ways first one is through increasing the concentration of TRIB3 inside the cell this phenomenon affects serine–threonine kinase Akt this enzyme also known as mTORC1 mammalian target of rapamycin C (Akt/mTORC1) axis, the inhibition this enzyme causes apoptosis mammalian cells .The second pathway of autophagy involves activation of AMPK abbreviated as adenosine monophosphate-activated kinase this activation happens CaMKKβ .Cannabinoids can also cause cell cycle arrest through activation of ERK1/2 this results in the release of an enzyme known as p27/KIP1 cyclin kinase inhibitor this enzyme inhibits the formation on cyclins resulting cell cycle arrest (Fonseca et al., 2017) .Due to the diversified function and complex distribution of receptors ECS has the potential to effect the signaling pathways involved in cancer , both CB1 and CB2 receptors are responsible antiproliferative and pro-apoptotic effect on cancer cells ,these receptors are Gi/o coupled seven-transmembrane domain receptor upon activation of alpha subunit of these receptors inhibition

of adenylate cyclase occurs , followed by inhibition of production of cAMP cyclic Adenosine Monophosphate and inhibition of PKA protein kinase A occurs, these events result to downregulation of gene transcription (Fonseca et al., 2017) . Cannabinoid induced apoptosis studies in various cell types including lymphoma B (Gustafsson et al., 2006) , endothelial(Rajesh et al., 2010) and epidermal cells (Llanos Casanova et al., 2003) showed that synthetic cannabinoid followed a common pathway that involves p3 8 MAPK phosphorylation followed by depolarization of membrane in mitochondria and activation of caspase to exert their apoptosis activity (Fonseca et al., 2017).Some agonists of CB1 and CB2 receptors have also shown anti-cancer effect due to increased synthesis of ceramide a pro-apoptotic factor, ceramide has a crucial role in inducing apoptosis in several kinds of cancerous cells for example in glioma cells upregulation of endoplasmic reticulum (ER) stress related gene which codes for ATF-4 (activating transcription factor 4) and CHOP C/EBP homologous protein and ER stress causing pseudo kinase these proteins are responsible for antiproliferative and pro-apoptotic effects , in leukemic cells ceramide induced apoptosis occurs through p38 mitogen-activated protein kinase(MAPK) activation (Javid et al., 2016). Agonists like AEA and CBD has some effects on lung cancer, they exert pro-apoptotic effect through regulation on ceramide. In lung cancer ceramide increases the level of expression of COX 2 cyclooxygenase 2 enzyme this event causes increased PGE2, pro-apoptotic prostaglandin E-2 synthesis (Hinz & Ramer, 2019). WIN55,212-2 (WIN) a synthetic cannabinoid agonis showed induced apoptosis in cerebellar granule cells through CB1 activation and reducing the level of the anti-apoptotic Bcl-xL inside the cell (Pozzoli et al., 2006) . Δ9-THC follows a different pathway from other cannabinoids. Δ9-THC induced apoptosis occurs through reduced intracellular level of ERK and PI3K/Akt in the survival pathways (Greenhough et al., 2007).

Through regulating balance between extracellular regulated kinase ERK, c-Jun N-terminal kinase JNK and p38 mitogen-activated protein kinase MAPK activities CB1 receptor can regulate cell growth and development , differentiation of cell and cell cycle arrest for example AEA halts the progression of cancerous cells in breast cancer through cAMP inhibition(de Petrocellis et al., 1998) .Apoptotic pathways may also be activating without modifying cell cycle stage for example in eCBS activates apoptotic pathway in glandular prostate cancer and endometrium cells (Orellana-Serradell et al., 2015).

Table 09: Experimental results of cannabinoids in different cancer cells.

The table below shows different molecular involvement of cannabinoid in different cancerous cells and affect those molecules exerts (Velasco et al., 2016) (Fallon et al., 2017). These data shows some molecule which is under investigation so may outcomes of experiment are yet to be published.

Tag	Illness	Involvement of molecules	Study strategy	Outcome
Pilot study	Glioblastoma	Δ9-THC	No result published yet.	No result published yet.
NCT02432612	This molecule is used in cancer with advanced stage	Sativex® developed by GW Pharmaceuticals in Britain was used	To assess the pharmacokinetic of Sativex® along with its tolerability profile an open-label trial was conducted	Study was inhibited before enrollment.

NCT01812603 this study was conducted	Used in grade 4 tumors like GBM or Glioblastoma	Sativex® developed by GW Pharmaceuticals in Britain was used	For assessment of pharmacodynamics, safety and tolerability of Sativex® an open-label trial with combination of temozolomide was used.	No result published yet.
NCT01812616	Used in grade 4 tumors like GBM or Glioblastoma	Sativex® developed by GW Pharmaceuticals in Britain was used	For assessment of pharmacodynamics, safety and tolerability of Sativex® placebo-controlled and double-blinded study was conducted in combination with temozolomide	No result published yet.
NCT01489826 this tag was used for this study.	Solid cancers	Dexanabinol synthetic cannabinoid	For assessment of pharmacokinetics open-label trial was conducted	No result published yet.
NCT01654497	Malignant brain tumors	Dexanabinol synthetic cannabinoid	For assessment of pharmacokinetics, safety and CNS activity open-label trials were conducted.	In progress
NCT02675842	Cancer in lungs	CBD: Δ9- THC smoke was used	For assessment of efficacy of cannabis in radiation therapy patients a placebo-controlled and a	In progress

			double-blind study was conducted.	
NCT02423239	Hepatocellular and pancreatic carcinomas	Dexanabinol s synthetic cannabinoid	For assessment of safety and efficacy open-label trials were conducted alongside with chemotherapies	In progress
ACTRN12616001036404 this tag was used	Any types of cancer cells	Δ9-THC and CBD was used.	For assessment of efficacy in reducing chemotherapy associated nausea and vomiting	In progress

Chapter 4

Symptomatic treatment and drugs binding to endocannabinoid receptors

Symptomatic treatment and Palliative care are a term that is used to treat patients with advanced, potentially ‘‘life-limiting’’ conditions like cancer, this class of treatment is used to ease the pain associated with morbid condition in cancer, usually palliative care option is given to patient and his family in advance stage of cancer to elevate to quality of life and with a goal of patient’s comfort. In cancer patients many cannabinoids’ molecules are effective for palliative care (Velasco et al., 2016). Generally opioids are used to treat cancer associated symptoms but opioids are associated with significant morbidity in long term treatment in palliative care from this perspective cannabinoids are far safer option for long term use in symptomatic treatment of cancer patient. Several studies suggested that there is a clear risk of fatal overdose prescription on the other hand several studies conducted on the adverse effect of cannabinoid medicines argue that adverse effects of cannabinoid-based medicines are minimal when compared to the improvement of the health condition of the patients (da Costa & de Carvalho, 2022). At present time many cannabinoid-based medicines are used to in the treatment of chemotherapy related adverse symptoms, for example dronabinol is used to treat nausea and vomiting associated with chemotherapy this drug molecule is 100% delta-9 THC, and is approved in 1986 by The Food and Drug Administration (Carter et al., 2011). Cannabinoids and ECS has a role in managing pain associated with oncology various clinical trials were also conducted for this purpose six studies were conducted. Out of six trials two double-blinded, randomized controlled and placebo-controlled study with nabixomol with trade name of Sativex has shown result shin numeric pain rating scale NRS greater than 4 less than 8 (Fallon et al., 2017), observational study has shown 50 percent reduction in pain and randomized controlled trials RCT data shows significant improvement (Gogichashvili, 2022). Based on the experimental data and side effect profile cannabinoids-based medicines can easily be used in treating cancer associated pains.

Chapter 5

Conclusion

Cancer is a hereditary disease that may result from mutations or alterations in the DNA of cells. Cancers vary greatly from one another, but they all share a basic trait. All cancers begin when normally functioning cells in the body develop abnormalities and begin to divide and spread uncontrollably. Treatments for cancer aim to halt or delay the progression of the disease. They eliminate cancerous tissue by eliminating it, destroying it, or preventing it from dividing further. Tumours or lumps may or may not form in response to some types of cancer. Cancer patients have a wide range of symptoms, undergo various diagnostic procedures, and have varying post-treatment survival prospects. Endocannabinoid system is very diversified system with its association with cell cycle of various cancerous cells by modulating CB receptors on cancer cells an anti-cancerous property can be observed. Ligands for this homeostasis system plays a great role in drug designing for advanced cancer treatment. Evidence shows cannabinoids both endo and phyto as well as synthetic ones play key role in symptom management of cancer, they also have the ability to slow down the cancer cell growth through interfering with different pathways in cell cycles. The anti-proliferating effect of cannabinoids can be used for target cancer therapy which requires further studies, cannabinoids show pro apoptotic effect and with its induced apoptotic effect it can be used for cancer treatment as a new class of drug, in dealing with cancer pain some properties of cannabinoids. Cancer medicines are associated with various adverse effects loss of appetite is one of them cannabinoids work as appetite stimulant and these molecules are associated with less side effects but the psychoactive effects of these molecules are the main concern for use of them in cancer treatment and management. By studying the structural activity relationship of these molecule and masking its psychoactive effects these molecules can be used as a lead compound for new drug discovery for cancer. As very recently discovered bodily system ECS requires further study and cannabinoids require further research work, based on the data it can be said that as a diversified disease cancer might need diversified molecules with multifunctional homeostatic system to fight.

Reference

Albertin, G., Fede, C., Sfriso, M. M., Macchi, V., Fede, C., Albertin, G., Petrelli, L., Sfriso, M. M., Biz, C., de Caro, R., & Stecco, C. (2016). Distribution of cannabinoid receptor 1 (CB1) and 2 (CB2) in subcutaneous tissue and fasciae Expression of the endocannabi-noid receptors in human fascial tissue. *European Journal of Histochemistry*, *60*, 2643. https://doi.org/10.13128/IJAE-17005

Axelrod1, J., & Felder1, C. C. (1998). Cannabinoid Receptors and Their Endogenous Agonist, Anandamide*. In *Neurochemical Research* (Vol. 23, Issue 5).

Behl, T., Makkar, R., Sehgal, A., Singh, S., Makeen, H. A., Albratty, M., Alhazmi, H. A., Meraya, A. M., & Bungau, S. (2022). Exploration of Multiverse Activities of Endocannabinoids in Biological Systems. In *International Journal of Molecular Sciences* (Vol. 23, Issue 10). MDPI. https://doi.org/10.3390/ijms23105734

Carter, G. T., Flanagan, A. M., Earleywine, M., Abrams, D. I., Aggarwal, S. K., & Grinspoon, L. (2011). Cannabis in palliative medicine: Improving care and reducing opioid-related morbidity. *American Journal of Hospice and Palliative Medicine*, *28*(5), 297–303. https://doi.org/10.1177/1049909111402318

Condie, R., Herring, A., Koh, W. S., Lee, M., & Kaminski, N. E. (1996). *Cannabinoid Inhibition of Adenylate Cyclase-mediated Signal Transduction and Interleukin 2 (IL-2) Expression in the Murine T-cell Line, EL4.IL-2**. http://www.jbc.org/

Crocq, M. A. (2020). History of cannabis and the endocannabinoid system. *Dialogues in Clinical Neuroscience*, *22*(3), 223–228. https://doi.org/10.31887/DCNS.2020.22.3/MCROCQ

da Costa, V. F. D. D., & de Carvalho, W. S. R. (2022). Use of Medicinal Cannabis for Palliative Care Patients: A Systematic Review. *Journal of Biosciences and Medicines*, *10*(09), 242–252. https://doi.org/10.4236/jbm.2022.109017

de Petrocellis, L., Melck, D., Palmisano, A., & Bisogno, T. (1998). The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation. In *Pharmacology* (Vol. 95). www.pnas.org.

di Marzo, V., Bifulco, M., & de Petrocellis, L. (2004). The endocannabinoid system and its therapeutic exploitation. In *Nature Reviews Drug Discovery* (Vol. 3, Issue 9, pp. 771–784). https://doi.org/10.1038/nrd1495

Elsohly, M. A. (n.d.). *Marijuana and the Cannabinoids Edited by*.

Fallon, M. T., Albert Lux, E., McQuade, R., Rossetti, S., Sanchez, R., Sun, W., Wright, S., Lichtman, A. H., & Kornyeyeva, E. (2017). Sativex oromucosal spray as adjunctive therapy in advanced cancer patients with chronic pain unalleviated by optimized opioid therapy: two double-blind, randomized, placebo-controlled phase 3 studies. *British Journal of Pain*, *11*(3), 119–133. https://doi.org/10.1177/2049463717710042

Fonseca, B. M., Teixeira, N. A., & Correia-da-Silva, G. (2017). Cannabinoids as modulators of cell death: Clinical applications and future directions. In *Reviews of Physiology, Biochemistry and Pharmacology* (Vol. 173, pp. 63–88). Springer Verlag. https://doi.org/10.1007/112_2017_3

Glass, M., Dragunow, † M, & Faull, R. L. M. (1997). *CANNABINOID RECEPTORS IN THE HUMAN BRAIN: A DETAILED ANATOMICAL AND QUANTITATIVE AUTORADIOGRAPHIC STUDY IN THE FETAL, NEONATAL AND ADULT HUMAN BRAIN*.

Gogichashvili, K. (2022). “Endocannabinoid System and Its Neuromodulatory Effects in Pain Management in Oncology Patients-A Systemic Review.” *Biomedical Journal of Scientific & Technical Research*, *45*(3). https://doi.org/10.26717/bjstr.2022.45.007213

Greenhough, A., Patsos, H. A., Williams, A. C., & Paraskeva, C. (2007). The cannabinoid Δ9-tetrahydrocannabinol inhibits RAS-MAPK and PI3K-AKT survival signalling and induces BAD-mediated apoptosis in colorectal cancer cells. *International Journal of Cancer*, *121*(10), 2172–2180. https://doi.org/10.1002/ijc.22917

Guindon, J., & Hohmann, A. G. (2011). *The endocannabinoid system and cancer: therapeutic implication LINKED ARTICLES*. https://doi.org/10.1111/bph.2011.163.issue-7

Gustafsson, K., Christensson, B., Sander, B., & Flygare, J. (2006). Cannabinoid receptor-mediated apoptosis induced by R(+)-methanandamide and Win55,212-2 is associated with ceramide accumulation and p38 activation in mantle cell lymphoma. *Molecular Pharmacology*, *70*(5), 1612–1620. https://doi.org/10.1124/mol.106.025981

Guzmán, M. (2003). Cannabinoids: Potential anticancer agents. In *Nature Reviews Cancer* (Vol. 3, Issue 10, pp. 745–755). European Association for Cardio-Thoracic Surgery. https://doi.org/10.1038/nrc1188

Hinz, B., & Ramer, R. (2019). Anti-tumour actions of cannabinoids. In *British Journal of Pharmacology* (Vol. 176, Issue 10, pp. 1384–1394). John Wiley and Sons Inc. https://doi.org/10.1111/bph.14426

Javid, F. A., Phillips, R. M., Afshinjavid, S., Verde, R., & Ligresti, A. (2016). Cannabinoid pharmacology in cancer research: A new hope for cancer patients? In *European Journal of Pharmacology* (Vol. 775, pp. 1–14). Elsevier B.V. https://doi.org/10.1016/j.ejphar.2016.02.010

Katona, I., & Freund, T. F. (2008). Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. In *Nature Medicine* (Vol. 14, Issue 9, pp. 923–930). https://doi.org/10.1038/nm.f.1869

Laezza, C., Pagano, C., Navarra, G., Pastorino, O., Proto, M. C., Fiore, D., Piscopo, C., Gazzerro, P., & Bifulco, M. (2020a). The endocannabinoid system: A target for cancer treatment. In *International Journal of Molecular Sciences* (Vol. 21, Issue 3). MDPI AG. https://doi.org/10.3390/ijms21030747

Laezza, C., Pagano, C., Navarra, G., Pastorino, O., Proto, M. C., Fiore, D., Piscopo, C., Gazzerro, P., & Bifulco, M. (2020b). The endocannabinoid system: A target for cancer treatment. In *International Journal of Molecular Sciences* (Vol. 21, Issue 3). MDPI AG. https://doi.org/10.3390/ijms21030747

Leo, L. M., & Abood, M. E. (2021). Cb1 cannabinoid receptor signaling and biased signaling. *Molecules*, *26*(17). https://doi.org/10.3390/molecules26175413

Llanos Casanova, M., Blázquez, C., Martínez-Palacio, J., Villanueva, C., Fernández-Aceñero, M. J., Huffman, J. W., Jorcano, J. L., & Guzmán, M. (2003). Inhibition of skin tumor growth and angiogenesis in vivo by activation of cannabinoid receptors. *Journal of Clinical Investigation*, *111*(1), 43–50. https://doi.org/10.1172/JCI200316116

Mechoulam, B. R., & Gaoni, Y. (n.d.). *Recent Advances in the Chemistry of Hashish*.

Murillo-Rodríguez, E. (2008). The role of the CB1 receptor in the regulation of sleep. In *Progress in Neuro-Psychopharmacology and Biological Psychiatry* (Vol. 32, Issue 6, pp. 1420–1427). https://doi.org/10.1016/j.pnpbp.2008.04.008

Orellana-Serradell, O., Poblete, C. E., Sanchez, C., Castellón, E. A., Gallegos, I., Huidobro, C., Llanos, M. N., & Contreras, H. R. (2015). Proapoptotic effect of endocannabinoids in prostate cancer cells. *Oncology Reports*, *33*(4), 1599–1608. https://doi.org/10.3892/or.2015.3746

Pertwee, R. G. (2001). Cannabinoid receptors and pain. In *Progress in Neurobiology* (Vol. 63). www.elsevier.com/locate/pneurobio

Pozzoli, G., Tringali, G., Vairano, M., D'Amico, M., Navarra, P., & Martire, M. (2006). Cannabinoid agonist WIN55,212-2 induces apoptosis in cerebellar granule CELLS via activation of the CB1 receptor and downregulation of bcl-xL gene expression. *Journal of Neuroscience Research*, *83*(6), 1058–1065. https://doi.org/10.1002/jnr.20794

Rajesh, M., Mukhopadhyay, P., Haskó, G., Liaudet, L., MacKie, K., & Pacher, P. (2010). Cannabinoid-1 receptor activation induces reactive oxygen species-dependent and -independent mitogen-activated protein kinase activation and cell death in human coronary artery endothelial cells. *British Journal of Pharmacology*, *160*(3), 688–700. https://doi.org/10.1111/j.1476-5381.2010.00712.x

Scotter, E. L., Abood, M. E., & Glass, M. (2010). The endocannabinoid system as a target for the treatment of neurodegenerative disease. In *British Journal of Pharmacology* (Vol. 160, Issue 3, pp. 480–498). John Wiley and Sons Inc. https://doi.org/10.1111/j.1476-5381.2010.00735.x

Silver, R. J. (2019). The Endocannabinoid System of Animals. *Animals*, *9*(9), 686. https://doi.org/10.3390/ani9090686

Suryadev, Y. Y., Kumar, V. A., Kumar, R., Vimal, Y., & Piyush, Y. (2017). *RESEARCHER's REFLECTION TARGETED CANCER THERAPY ARTICLE INFO ABSTRACT* (Vol. 1, Issue 1). http://pgi.edu.inAvailableonlineatPgi.edu.in

Svíženská, I., Dubový, P., & Šulcová, A. (2008). Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures - A short review. In *Pharmacology Biochemistry and Behavior* (Vol. 90, Issue 4, pp. 501–511). https://doi.org/10.1016/j.pbb.2008.05.010

Ueda, N., Tsuboi, K., Uyama, T., & Ohnishi, T. (2011). Biosynthesis and degradation of the endocannabinoid 2-arachidonoylglycerol. *BioFactors*, *37*(1), 1–7. https://doi.org/10.1002/biof.131

Vecera, L., Gabrhelik, T., Prasil, P., & Stourac, P. (2020). The role of cannabinoids in the treatment of cancer. In *Bratislava Medical Journal* (Vol. 121, Issue 1, pp. 79–95). Comenius University. https://doi.org/10.4149/BLL_2020_012

Velasco, G., Sánchez, C., & Guzmán, M. (2012). Towards the use of cannabinoids as antitumour agents. In *Nature Reviews Cancer* (Vol. 12, Issue 6, pp. 436–444). https://doi.org/10.1038/nrc3247

Velasco, G., Sánchez, C., & Guzmán, M. (2016). Anticancer mechanisms of cannabinoids. In *Current Oncology* (Vol. 23, pp. S23–S32). Multimed Inc. https://doi.org/10.3747/co.23.3080

Salehin_project Summer 2022

ORIGINALITY REPORT

5%	4%	2%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	epdf.pub Internet Source	2%
2	B. M. Fonseca, N. A. Teixeira, G. Correia-da-Silva. "Chapter 3 Cannabinoids as Modulators of Cell Death: Clinical Applications and Future Directions", Springer Nature, 2017 Publication	1%
3	www.bps.ac.uk Internet Source	<1%
4	Submitted to University of Nicosia Student Paper	<1%
5	docksci.com Internet Source	<1%
6	link.springer.com Internet Source	<1%
7	pure.manchester.ac.uk Internet Source	<1%
8	www.frontiersin.org Internet Source	<1%