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REVIEW ARTICLE

Endocannabinoid System: A Multi-Facet Therapeutic Target

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Abstract: Cannabis sativa is also popularly known as marijuana. It has been cultivated and used by man for recreational and medicinal purposes since many centuries. Study of cannabinoids was at bay for very long time and its therapeutic value could not be adequately harnessed due to its legal status as proscribed drug in most of the countries.

The research of drugs acting on endocannabinoid system has seen many ups and downs in the recent past. Presently, it is known that endocannabinoids has role in pathology of many disorders and they also serve "protective role" in many medical conditions. Several diseases like emesis, pain, inflammation, multiple sclerosis, anorexia, epilepsy, glaucoma, schizophrenia, cardiovascular disorders, cancer, obesity, metabolic syndrome related diseases, Parkinson's disease, Huntington's disease, Alzheimer's disease and Tourette's syndrome could possibly be treated by drugs modulating endocannabinoid system. Presently, cannabinoid receptor agonists like nabilone and dronabinol are used for reducing the chemotherapy induced vomiting. Sativex (cannabidiol and THC combination) is approved in the UK, Spain and New Zealand to treat spasticity due to multiple sclerosis. In US it is under investigation for cancer pain, another drug Epidiolex (cannabidiol) is also under investigation in US for childhood seizures.

Rimonabant, CB_1 receptor antagonist appeared as a promising anti-obesity drug during clinical trials but it also exhibited remarkable psychiatric side effect profile. Due to which the US Food and Drug Administration did not approve Rimonabant in US. It sale was also suspended across the EU in 2008. Recent discontinuation of clinical trial related to FAAH inhibitor due to occurrence of serious adverse events in the participating subjects could be discouraging for the research fraternity. Despite some mishaps in clinical trials related to drugs acting on endocannabinoid system, still lot of research is being carried out to explore and establish the therapeutic targets for both cannabinoid receptor agonists and antagonists. One challenge is to develop drugs that target only cannabinoid receptors in a particular tissue and another is to invent drugs that act selectively on cannabinoid receptors located outside the blood brain barrier. Besides this, development of the suitable dosage forms with maximum efficacy and minimum adverse effects is also warranted. Another angle to be introspected for therapeutic abilities of this group of drugs is non-CB_1 and non-CB_2 receptor targets for cannabinoids.

In order to successfully exploit the therapeutic potential of endocannabinoid system, it is imperative to further characterize the endocannabinoid system in terms of identification of the exact cellular location of cannabinoid receptors and their role as "protective" and "disease inducing substance", time-dependent changes in the expression of cannabinoid receptors.

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INTRODUCTION

Endocannabinoid System

Two types of cannabinoid receptors have been identified as CB_1 and CB_2. They are G protein coupled receptors. These receptors are not only activated by endogenous substances known as endocannabinoids but also by cannabis derived substances and synthetic agonists. Recently some non-CB_1 and non CB_2 receptor targets for cannabinoids were discovered such as GPR55 [1-3] and TRPV1 [4]. Endo-cannabinoids, cannabinoid receptors, enzymes responsible for synthesis and degradation of endocannabinoids all together constitute the endocannabinoid system [5, 6].

At least five endogenous substances known to have affinity for cannabinoid receptors are produced in human brain. They are termed as endocannabinoids. The prominent endocannabinoids are anadamide and 2-arachidonoyl glycerol [7]. Endocannabinoids are not stored in vesicles; they act "on demand". Endocannabinoids are retrograde transmitters [8]. They are released from the postsynaptic cell and act on the presynaptic cell. They reduce the amount of presynaptic neurotransmitter release. Common feature of all the endocannabinoids is to suppress pain sensitivity.

Out of all the endocannabinoids discovered till date, anandamide is the most widely studied [7]. It has ability to

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bind to both CB_1 and CB_2 receptors. It also binds to transient receptor potential vanilloid 1 receptor (TRPV1) [9]. Cellular coexistence of cannabinoid receptors and TRPV1 would lead to enhanced biological activity mediated by agonists of these receptors [10].

Localization of Cannabinoid Receptors

Discovery of endocannabinoids and CB_1 and CB_2 receptors evoked interest of the researchers to determine and classify the location of these receptors in human body. It is known that CB_1 receptors are expressed on the axon terminals of central and peripheral neurons. In these neurons they inhibit the release of various neurotransmitters such as acetylcholine, norepinephrine, dopamine, 5-hydroxytryptamine, glutamate, GABA [6, 8, 11].

In CNS, CB_1 receptors are found abundantly in hippocampus, the area of brain responsible for short-term memory and in the amygdala, the part of brain involved with memory of fear, pain and emotional control [12]. The site of existence of CB_1 receptors in the central nervous system justify several effects produced Δ-9 tetrahydrocannabinol, the major psychoactive component of cannabis, for example cognition and memory, alteration in the control of motor function, and induction of analgesia. Peripherally they are found in adipocytes [11], liver [13], pancreas [14] and skeletal muscles [15]. They have been also located in some non-neuronal cells including the immune cells [16, 17]. CB_2 receptors are mainly expressed in immune cells like microglia, osteoclasts and osteoblasts [18].

The detailed location and function of CB_1 and CB_2 receptors are shown in Table **1**. Activation of these receptors also leads to inhibition of neurotransmitters. Besides this, they also affect the cytokine secretion by the immune cells. CB_2 receptors are also located in some central and peripheral neurons but their role in neurons is not yet well established [19].

Signaling Pathways of Cannabinoids

The CB_1 negatively regulates neurotransmitter release by inhibiting the phosphorylation of A-type potassium channels. Continuous potassium currents from unphosphorylated A-type potassium channels may prevent neurotransmission. Besides this, it also leads to inhibition of N-type calcium channels by CB_1 through direct interaction with the inhibitory G protein (Gi/o). This CB_1- mediated restriction of neurotransmission *via* potassium and calcium channels accounts for cognitive impairment and sedative-like effects experienced by marijuana users.

Table 1. Location and function of cannabinoid - CB_1 and CB_2 receptors.

Location	Function
Central CB_1 Receptors	
Hippocampus	Memory storage
Cerebellum	Coordination of motor function, posture, balance
Basal ganglia	Movement control
Hypothalamus	Thermal regulation, neuroendocrine release, appetite
Spinal cord	Nociception
Cerebral cortex	Emesis
Peripheral CB_1 Receptors	
Lymphoid tissue	Cell-mediated and innate immunity
Vascular smooth muscle cells	Control of blood pressure
Duodenum, ileum, myenteric plexus	Control of emesis
Lung smooth muscle cells	Bronchodilation
Eye ciliary body	Intraocular pressure
Peripheral CB_2 Receptors	
Lymphoid tissue	Cell-mediated and innate immunity
Peripheral nerve terminals	Peripheral nervous system
Retina	Intraocular pressure
Central CB_2 Receptors	
Cerebellar granule cells mRNA	Coordination of motor function

CB-Cannabinoid.

Both CB_1 and CB_2 receptor are primarily coupled with inhibitory G proteins Gi and Go [6]. Stimulation of CB_1 receptors leads to inhibition of adenyl cyclase and activation of mitogen- activated protein (MAP) kinase. CB_1 receptors are also coupled with ion channels through Gi/o proteins.

The CB_1 receptors negatively regulate the neuro-transmitter release by inhibiting the phosphorylation of A-type potassium channels. Continuous potassium currents from unphosphorylated A-type potassium channels may prevent neurotransmission. Besides this, it also leads to inhibition of N-type calcium channels by CB_1 through direct interaction with the inhibitory G protein (Gi/o). Stimulation of CB_2 receptors results in similar signaling events but the modulation of ion channels mediated by them is more variable [6].

There are several reports that CB_1 receptors can also stimulate adenylyl cyclase *via* G_s, induce receptor-mediated Ca^{2+} fluxes and stimulate phospholipases [20]. Cannabinoid receptors are known to modulate several other signaling extracellular signal-regulated kinase ERK, c-Jun-NH2-kinase and the ceramide pathway [20].

Metabolism of Endocannabinoids

The biosynthesis of endocannabinoid takes place on demand in response to elevations of intracellular calcium. The biosynthesis of anandamide occurs through pathways involving *N*-acylphosphatidylethanolamide-phospholipase D (NAPE-PLD), a secretory PLA and PLC. The synthesis of 2-arachidonoylglycerol is mediated through the action of selective enzymes like phosphatidic acid phsophohydrolase, diacylglycerol lipase (DAGL), phosphoinositide-specific PLC (PI- PLC) and lyso-PLC. The actions of endocannabinoids are tightly regulated by enzymatic degradation.

After producing their effect in the synaptic cleft (inhibiting release of neurotransmitters) they are removed by putative membrane transporter or facilitated diffusion from the site of action by cellular uptake mechanisms. They are metabolized intracellularly. They are degraded by two, fatty acid amidohydrolase (FAAH) and monoacyl glycerol lipase (MAGL).

The distribution pattern of cannnabinoid receptors, FAAH, MAGL is somewhat similar in some parts of CNS [21]. Other enzymes that can participate in their metabolism are cyclooxygenase-2, lipoxygenases, and cytochrome P^{450} [22].

Development of inhibitors of the enzymes involved in the endocannabinoid metabolism is emerging as attractive therapeutic target. Recent mishap in Phase I clinical trial of a FAAH inhibitor BIA10-2474 came as a big setback for researchers. This trial had to be discontinued due to hospitalization of the six participants, out of them one was dead and other four suffered irreversible brain damage [23, 24]. Other clinical trials that are conducted on FAAH inhibitors are Merck's MK-4409, Pfizer's PF-04457845, and Vernalis' V158866 [23].

Cannabinoid Agonists

The discovery and distribution pattern of cannabinoid receptors evoked the interest of scientists not only to isolate the active principles in cannabis but also to synthetically develop selective CB_1 and CB_2 agonists. In 1981, first cannabinoid agonist nabilone was introduced in the market. It is synthetic analogue of Δ-9 THC. It is used for chemotherapy -induced nausea and vomiting. Later in 1985, Δ-9 THC itself under the name of dronabinol was introduced in the market for management of chemotherapy-induced nausea and vomiting. In 1992, dronabinol made its place in market as an appetite stimulant as well. Combination of D9-THC and cannabidiol is prescribed for the symptomatic relief of neuropathic pain in adults with multiple sclerosis and as an adjunctive analgesic treatment for adult patients with advanced cancer.

With the advancement of research techniques and knowledge of therapeutic potential of cannabinoids, more and more work was carried out on cannabinoid agonists and it is suggested through various researches that cannabinoid receptor agonists may have possible role in relief of pain associated with cancer, multiple sclerosis, cardiovascular disorders like atherosclerosis; in management of tics and behavioral issues seen in patients with Tourette's syndrome, in anxiety disorders, ADHD and depression, to inhibit the growth of malignant tumors by inhibiting angiogenesis, in gastrointestinal disorders, management of tardive dyskinesia induced in psychiatric patients by neuroleptic drugs, management of glaucoma, cough and cholestatic pruritus [25, 26].

The cannabinoid receptor drugs currently available in the market lack specificity and thus produces numerous central adverse effects such as acute psychotic episodes, euphoric mood changes, exacerbation of schizophrenic psychosis in predisposed persons, impaired cognitive and psychomotor performance, tachycardia and hypotension [27]. Fride in 2004 showed that the therapeutic and physiological effects of cannabinoid agonists are dependent on the route and duration of its administration [28].

Another issue with these drugs is that on long-term use tolerance may develop to many effects produced by them. This tolerance seems to be pharmacodynamic in nature mainly either due to internalization of the receptors or due to reduction in receptor protein synthesis [29, 30].

Cannabinoid Antagonists

Hyperactivity of endocannabinoid system plays a vital role in etiology of various diseases. Thus, a lot of research is being carried out to develop drug molecules that can act as antagonists at these receptors and provide salutary gain in these pathological conditions. Selective antagonists for CB_1 and CB_2 receptors have been developed.

CB_1 receptor antagonists have therapeutic potential to treat overweight/obesity, obesity-related cardiometabolic disorders, and substance abuse.

Rimonabant was the first cannabinoid antagonist that was launched in the market in Europe in 2006 for the management of obesity. It is inverse agonist/ antagonist at CB_1 receptors. Rimonabant was withdrawn in 2008 from the EU market owing to increased incidences of depression and suicidal tendencies in patients using this drug. These adverse

events associated with Rimonabant were assigned to its inverse agonistic activity. Thereafter the research in this field was centralized on discovering the neutral antagonists and developing drugs that have restricted activity of blocking CB_1 receptors in the peripheral tissues.

CB_2 antagonists possibly have potential as immuno-modulatory and anti-inflammatory drugs [31]. Drugs acting on cannabinoid receptors which are approved for sale are shown in Table **2**.

FUTURE STRATEGIES

Cannabis played an important role in medical history of ancient times. In the 19^{th} and 20^{th} century when medical prescribing came into practice, the use of cannabis was limited to illicit practice and self-medication. But in the last two decades the therapeutic role of cannabis and cannabinoids has been suggested in so many conditions that it gives an impression that it could be a revolutionary group of drugs in the history of medicine. Drugs affecting the cannabinoid system as fore mentioned might be useful in many conditions critical to human health, but the numerous adverse effects attached to this therapy limit its therapeutic use. Some of the possible uses of drugs acting through cannabinoid system are given in Table **3**.

But as mentioned earlier in the article, the legal position of cannabis around the world and liability of psycotrophic effects attached to it raise doubts on its therapeutic value. Another factor that restricted the study on this substance was that the most common route known to administer this drug was smoking and this route of drug delivery is not very well accepted in medicine. Thus, all these factors limited the research work done on this highly valuable therapeutic agent and the work carried out on it was scanty for many years.

A number of strategies have been suggested to exploit the beneficiary effects of cannabinoid system with minimal adverse effects. Some of them have mentioned below:

Development of Drugs that cannot Cross Blood Barrier

Majority of adverse effects produced by this group of drugs have been ascribed to the central action produced by them. On the other hand, it has been established through various studies that the beneficial effects produced by these drugs are due to activation of CB_1 and CB_2 receptors outside the blood brain barrier. Pain relief, inhibition of cancer cell proliferation, management of some cardiovascular and gastrointestinal conditions are some important targets for peripheral cannabinoid drugs [32].

Thus, development of drug molecules which can be primarily restricted to peripheral tissues and are incapable of crossing blood brain barrier is the prime focus for research in this field at present. In order to develop drugs selectively acting on peripheral cannabinoid receptors, some of the synthetic approaches that are being tried are designing highly polar, water soluble with polar surface area [33]. There are studies that claim that efflux *via* transporters such as P-gp and BCRP can limit the brain penetration of CB1 receptor antagonists, and that this property could be used in the development of peripheral antagonists [34].

If success is attained in developing CB_1 receptor antagonists with poor ability to cross blood brain barrier, it could possibly be a landmark in management of obesity and other metabolic disorders. Another alternative is to develop peripherally restricted selective inhibitors (FAAH and MAGL inhibitors) of endocannabinoid metabolism. The advantage of such metabolism inhibitors over cannabinoid receptor agonists is that the elevation in endocannabinoid levels would be achieved only at the peripheral physiological sites of endocannabinoid production and release.

Development of Tissue Selective Drugs

One mode of achieving tissue selective action of a drug is to deliver the drug through such a route that its impact on other tissue is minimized**.** Lot of work in being carried out to study the effect of intrathecally delivered cannabinoid drugs [35, 36].

The aim is to target the CB_1 receptors within the spinal cord to alleviate pain of neuropathic origin or acute inflammatory pain. The pain localized to the skin surface is targeted by delivering the cannabinoid drugs using skin patches. This strategy is based on two findings. One is that the CB_1 and CB_2 receptors are found in the mast cells, macrophages, epithelial cells of hair follicles, cutaneous nerve fibers. Another finding that promotes this targeted delivery in the skin is the established fact the stimulation of the cannabinoid receptors in skin has analgesic effect in acute, inflammatory and neuropathic pain [37-40].

Efforts are being made to obtain target delivery of the cannabinoid drugs. Preclinical studies on administering

Table 2. Approved drugs which act on cannabinoid - CB_1 and CB_2 receptors.

Drug	Receptors	Therapeutic Application
Nabilone	CB_1 agonist	Suppression of nausea and vomiting produced by chemotherapy
Δ9-THC	CB_1/$5HT_3$ agonist	Anti-emetic and as an appetite stimulant
Combination of Δ9-THC and cannabidiol (Sativex)	CB_1 and CB_2 agonist	Symptomatic relief of neuropathic pain in adults with multiple sclerosis and as an adjunctive analgesic treatment for adult patients with advanced cancer
Rimonabant	CB_1 antagonist/inverse agonist	Anti-obesity drug

THC-Tetrahydrocannabinol, CB_1-Cannabinoid, $5HT_3$-5-Hydorxytryptamine.

drugs through skin patch, microdialysis [41, 42], implants [43], direct injections in the affected site [44] have given promising results in preliminary studies.

Stimulating Upregulated Receptors

As a defense mechanism of body, in certain disorders there is upregulation of cannabinoid receptors. If these upregulated receptors are activated, the progression of the disease might slowdown [5]. Another approach is to use partial agonist in disorders that triggers upregulation of cannabinoid receptors as a protective mechanism.

Endocannabinoids and exogenous cannabinoids are known to cause CB_1 receptor up-regulation in hepatocytes and T cells, respectively [45, 46]. High fat diet also causes CB_1 receptor upregulation in liver and adipose cells and the long term blockage of CB_1 receptors in these tissue lead to counter the upregulation [47]. The endocannabinoids are also usually found to be increased along with the increase in cannabinoid receptors in many disease states [48] CB_1 and CB_2 receptors are up-regulated: in peripheral and central sensory pathways in animal models of neuropathic pain [49], in multiple human cancers [50], hepatic CB_1 and CB_2 receptors up-regulated in cirrhosis in humans [51]. This autoinduction may have therapeutic implication if the mechanisms of upregulation are determined. There are studies which indicate that this up-regulation is mediated in part by Trk/MAPK pathways and glucocorticoid receptors [52, 53].

Table 3. Potential therapeutic targets.

Pharmacological Actions	Therapeutic Effects
Bronchodilation	Bronchial asthma
Antiemetic	Prevention of nausea/vomiting caused by anticancer drugs
Appetite stimulation	Palliative care for anorexia caused by opioids, antiviral drugs, AIDS-related illness or terminal cancer
Analgesia	Cancer pain, post-operative pain
Decreased spasticity/ataxia/muscle weakness	Multiple sclerosis, cerebral palsy, spinal injuries
Decreased intraocular tension	Glaucoma
Decrease in blood pressure	Hypertension

Table 4. Some non CB_1 and non CB_2 targets for cannabinoid agonists.

Receptor/ Ligand Gated Ion Channels	Response
Glycine receptors	Ion current decreased
NR1A containing NMDA channels	Ion current potentiation
5- HT_2 receptors	5-HT binding increased induced
5-HT_3 receptors	Ion current decrease induced
Central TRPV1-like receptors	Ion current decreased
GPR55	Increased release of calcium from intracellular stores
Voltage-Gated Ion Channel	
T-type Ca^{2+} channels	Ion current decreased
N-type Ca^{2+} channels	Ion current decreased
Na^{+} channels	Ion current decreased
Ca^{+} activated K^{+} channels	Ion current potentiation
Other type of voltage gated K^{+} channels	Ion current decreased
Sites of Neuronal Transporters	
Dopamine transporter	Synaptosomal uptake potentiation /decreased
Noradrenaline transporter	Synaptosomal uptake potentiation
5-HT transporter	Synaptosomal uptake potentiation /decreased

NR1A-NMDA receptors, NMDA-N-Methyl-D-aspartate, 5HT-Hydorxytryptamine, TRPV1-Transient Receptor Potential Vanilloid-1.

Administration of Trk agonists or glucocorticoids could contribute to the upregulation, although at this point these are only speculations and further elucidation is required to establish such drug designs. In disease conditions where cannabinoid receptors are protective, enhancement of up-regulation could be used as alternatives to agonists. On the other hand, if the upregulated receptors are contribute to the etiology of disease, the inhibition of up-regulation could be an alternative to antagonists [48, 51].

Targeting CB_2 Receptors

Development of drug molecules that are selective for CB_2 receptors will eradicate the danger of occurrence of central CB_1 receptor mediated adverse effects. Selective CB_2 receptor agonists have demonstrated their value in persistent inflammatory pain, acute pain, post-operative pain, neuropathic pain and cancer pain in animal models.

Besides this CB_2 receptor agonists have possible therapeutic application in some neurodegenerative [54, 55], immunological, inflammatory, cardiovascular [56-59], hepatic [60-62], renal [63] and bone disorders [64].

Additional Non CB_1 and Non CB_2 Targets for Cannabinoid Agonists

Cannabidiol, an endocannabinoid, produces its effect independent of CB_1 and CB_2 receptors and it does not produce psychoactivity. This raised interest in finding non-CB_1 and non-CB_2 targets for cannabinoids. Some of these non-CB_1 and non-CB_2 targets are given in Table **4**.

Another approach towards research could be development of drugs that inhibits either or both processes of cellular uptake and intracellular metabolism of endo-cannabinoids. Several such possibilities have been tried in experimental models [65, 66].

Targeting Enzymes Involved in Metabolism of Endocannabinoids

One of the major advances in the recent research is the generation of Fatty acid amide hydrolase (FAAH) inhibitors [67, 68]. FAAH hydrolyses the endocannabinoids with amide bonds including anandamide. Inhibition of FAAH would lead to extended endocannabinoid activity at its site of synthesis, resulting in tissue selective activation of CB_1 receptors. This enhanced endocannabinoid activity is suggested to be useful in the treatment of several clinical conditions. At present lot of research is being carried out to establish their role in management of neupathetic pain.

As discussed earlier, the recent discontinuation of phase I clinical trial with a FAAH inhibitor is discouraging. However, the exact cause of mishap is not yet clear and the adverse events caused in human subjects could be due to human error and not because of drug molecule.

Thus, further research on FAAH inhibitors could lead to fruitful results.

CONCLUSION

The endocannabinoid system is a key player in several physiological and pathological mechanisms in humans.

Drugs that enhance the activity of endocannabinoids like cannabinoid receptor agonists, agents modifying cannabinoid transport or inhibiting their metabolism has capacity to be used as analgesics, hypnotics, antiemetics, antihypertensives, antiasthmatics, antiepileptics, neuroprotectives, immuno-modulatory, anti-inflammatory, alcohol withdrawal, eating disorders, treatment of glaucoma, spasticity and other motor disorders. CB_2 receptor modulation has possible role in analgesia, bone growth, atherosclerosis and hepatic fibrosis. CB_1 receptor antagonist is known to have role as anti-obesity, other cardiometabolic disorders and substance abuse.

If all these claimed therapeutic applications of the drugs modulating cannabinoid system, comes in clinical practice, it would bring rewarding changes in pharmacotherapeutics of many disease states.

The endocannabinoid system is being thoroughly investigated for all the appealing therapeutic potentials mentioned earlier in the article. From the research carried so far, it is clear that the therapeutic value of endocannabinoid system can be achieved by manipulation of levels of endogenous cannabinoids. The levels of endocannabinoids can be altered either by exogenous targeting of the cannabinoid receptors or by influencing their synthesis, transport, release and degradation.

One of the drawbacks of investigating cannabinoids is their classification as substances of abuse. Besides this, there are certain other limitations due to which the exact potential of this multi-facet therapeutic target is not yet fully exploited. Most of the studies conducted so far are in preclinical settings. The results of translation for these preclinical outcomes into the clinical settings are not very positive due to inter-species variations in endocannabinoid signaling in animals and humans. Genetic polymorphism is common feature of the components of endocannabinoid system. Moreover, the presence of limited number of receptor subtypes and endogenous ligands and their wide distribution throughout the body make the systemic and organ-specific targeting difficult.

Endocannabinoid system is deeply involved in basic physiological functions. They act through various signaling pathways and they have numerous feedback and regulation processes attached to it. Thus, selective targeting is difficult to attain. The challenge now is not only to expand the current knowledge about endocannabinoid system and their exogenous agonists, antagonists and inverse agonists but also to characterize their non CB_1 and non CB_2 sites of action. Another important aspect is to continue research to further understand the role of endocannabinoids in physiological and pathophysiological conditions. This would warrant the development of improved and advanced research tool, such as more reliable and specific antibodies, bioassay techniques, etc.

Despite the fact that one of the drugs acting on endocannabinoid system was withdrawn and for another the clinical trial had to be stopped, it is still expected that that the next few years will be promising for endocannabinoid system as an appealing target for drug development.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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