ELSEVIER

Targeting the endocannabinoid system: future therapeutic strategies

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The endocannabinoid system (ECS) is involved in many physiological regulation pathways in the human body, which makes this system the target of many drugs and therapies. In this review, we highlight the latest studies regarding the role of the ECS and the drugs that target it, with a particular focus on the basis for the discovery of new cannabinoid-based drugs. In addition, we propose some key steps, such as the creation of a cannabinoid–receptor interaction matrix (CRIM) and the use of metabolomics, toward the development of improved and more specific drugs for each relevant disease.

Introduction

The ECS is an important physiological system that is involved in some of the main, albeit basic, functions of the human body. It is a versatile system that acts as a broad-spectrum modulator. Its components utilize basic biological mechanisms and have numerous interactions that affect the physiology and pathology of the central (CNS) and peripheral nervous systems [1]. The ECS is involved in various processes, including brain plasticity, learning and memory, neuronal development and cellular fate, nociception, inflammation, appetite regulation, digestion, suckling in the newborn, metabolism, energy balance, thermogenesis, motility, sleep–wake cycle, regulation of stress and emotions, and addiction. This extensive involvement in such vital processes makes it a key target for potential therapies.

The ECS includes two major G-protein-coupled receptors (GPCR): CB_1, mainly expressed in brain structures and CB_2, whose expression is more limited to immune system cells. Recent studies revealed that the vanilloid type 1 receptor (TRPV1) and G-protein-coupled receptor 55 (GPR55) act as putative cannabinoid receptors (CRs) that are directly related to the ECS. The activation of CB_1 and CB_2 is mediated by two endogenous ligands, anandamide (AEA) and 2-arachidonoyl-glycerol (2-AG), which are considered to be the main endocannabinoids (ECs) [2]. These ECs, together with various enzymes involved in the biosynthesis and/or degradation of endogenous lipid ligands, comprise the ECS (Fig. 1). This ECS family continues to expand as research progresses.

The CB_1 receptor is the most numerous metabotropic receptor in the mammalian brain. Its ligand, 2-AG, and possibly also AEA work as a retrograde messengers that modulate synaptic activity not only through neuronal positioning, but also via non-neuronal cells [3,4]. The main basic mechanisms triggered by CRs are mediated by G proteins, mainly $G_{i/o}$, resulting in the inhibition of the AC/cAMP cascade and voltage-gated calcium channels, as well as the stimulation of inwardly rectifying potassium currents and promotion of mitogen-activated protein kinase (MAPK) activity [2,5]. However, depending on the availability of the subunits of G proteins, dimerization with other proteins in particular cellular environments can result in binding to different G proteins, such as G_s or $G_{q/11}$ [6], which could have implications for the signal-regulating mechanism(s).

Overall, it has been shown the ECS has a homeostatic role in various physiological and pathological conditions. Indeed, its characteristic on-demand biosynthesis and release makes it a system capable of adapting quickly to changing conditions. Thus, the adaptive response of the ECS has evolved to restore homeostasis; however, in some diseases, this response contributes to disease symptoms or progress: for instance, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS), and epilepsy are examples of neurological disorders in which the ECS is altered [2].

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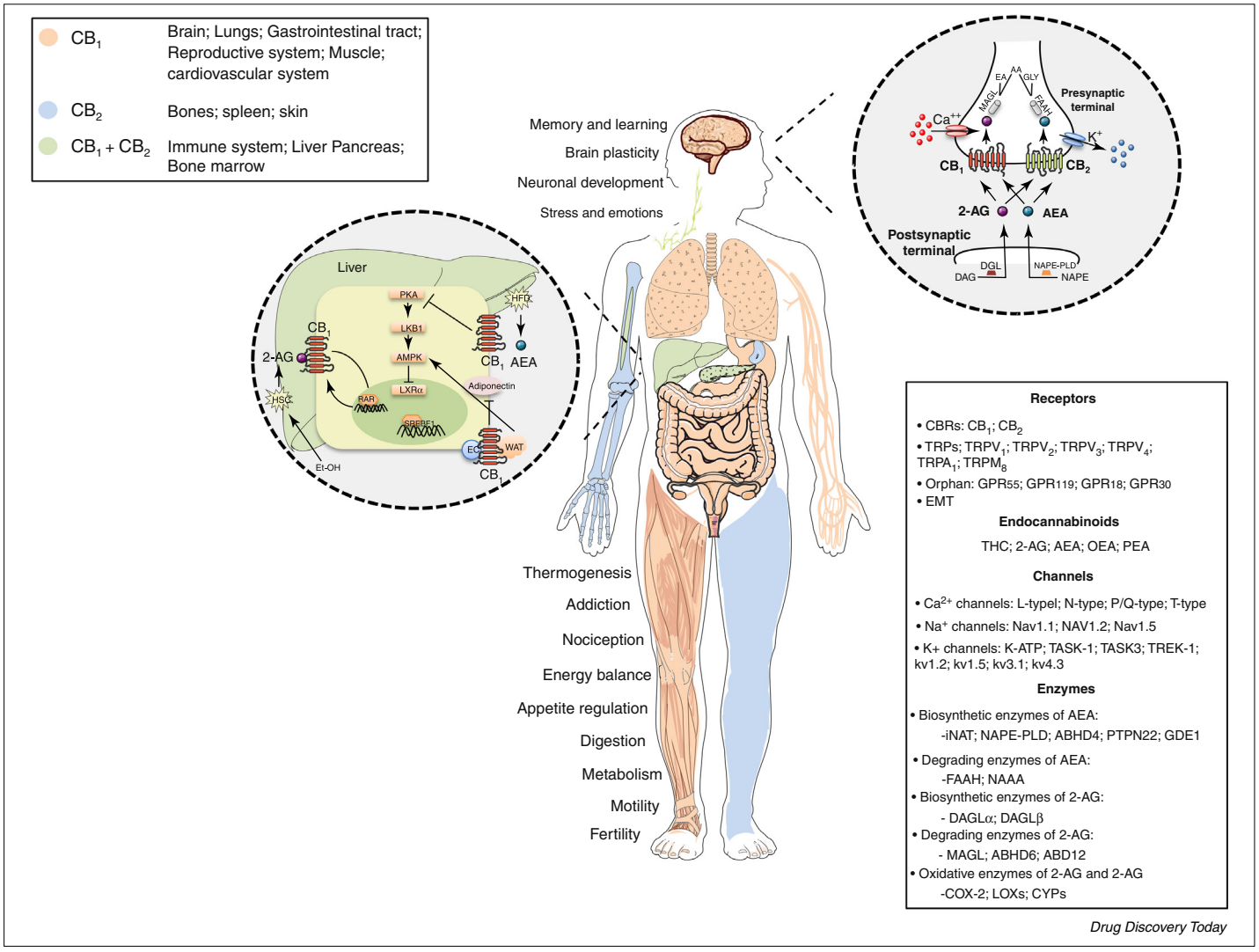

FIGURE 1

The endocannabinoid system: its functions, distribution, and components. Adapted from Servier Medical Art

Thus, there is a current focus on the development of new ligands that can be administrated exogenously to mimic the protective effects of ECs. However, this research field is becoming increasingly complex as more putative endogenous ligands, receptors, targets, sites of EC interactions, and enzymes involved in this system are identified [7]. This ever-increasing family was defined recently as the 'endocannabinoidome' [2], and we suspect that this is only the tip of the proverbial iceberg.

It has been shown that ligands such as plant-derived cannabinoids (commonly known as phytocannabinoids) and aminoalkylindoles are involved in the activation of CRs [2]. Over the past two decades, CB_1 and CB_2 receptor agonists have been used in clinic for the suppression of nausea and emesis, as appetite stimulants, and as analgesics for neuropathic pain in adults with MS and advanced cancer. In recent years, because of the adverse effects of the synthetic CB_1 inverse agonist SR141716A (Rimonabant; Acomplia), research attention has focused on a second generation of synthetic cannabinoids, including CB_1 neutral antagonists such as AM4113, CB_1/CB_2 agonists that do not cross the blood–brain barrier, and ligands for CB_2 receptors, mainly because such compounds are thought to have improved benefit:risk ratios compared with centrally active CB_1 receptor agonists [8,9].

Despite the importance of the ECS in various physiological processes, it was not studied in detail until the early 1960s, when the major psychoactive constituent of *Cannabis sativa*, Δ-9-tetrahydrocannabinol (THC), was discovered. THC is one of at least 113 phytocannabinoids that have been identified so far from *C. sativa* [10]. For 20 years, THC was thought to be a compound characterized by nonspecific activity. However, during the mid-1980s, pharmacological and chemical research by Allyn Howlett's group showed that cannabinoids inhibited cAMP accumulation in neuronal cells, suggesting the existence of a specific site of action involved in this inhibition [1]. As a result, the ECS has become one of the main targets for the design of drugs that could act as therapeutic agents in pathological processes. In addition to THC, *C. sativa* contains other cannabinoids and terpenes that enhance the beneficial effect of Δ^9-THC or attenuate its adverse effects [10].

Some of these cannabinoids are also known to activate CRs, although more research is needed to establish the specific

mechanism of action because the observed patterns of activation differ depending on the compounds used: THC, delta-8-tetrahydrocannabinol (Δ^8-THC), and cannabinol (CBN) have been found to be CB_1- and CB_2-activating compounds; delta-9-tetrahydrocannabivarin (THCV) acts as a CB_1 antagonist and CB_2 agonist; and the sesquiterpene caryophyllene (E)-BCP, also present in other plant families, activates CB_2 but not CB_1 receptors. In addition, these compounds are effective at activating or blocking transient receptor potential (TRP) cation channels, GPR55 receptors, and peroxisome proliferator-activated receptors (PPARs), among others [8]. Thus, the additional CR-independent actions of these compounds require further research attention.

The capacity of other *C. sativa* constituents to activate CB_1 and CB_2 receptors is the subject of ongoing research. Thus far, no other phytocannabinoid has been reported to activate either the CB_1 or CB_2 receptor with significant potency, although some are able to modulate 5TH1A and α(2)-adrenoceptors, TRP cation channels, and so on [8,11]. Compounds such as cannabidiol (CBD), cannabidiolic acid (CBDA), and cannabigerol (CBG) have shown potential therapeutic action in the suppression of chemotherapy-induced nausea and vomiting or as analgesics [8]. However, further investigations are required to identify the full range of phytocannabinoids in *C. sativa*, their metabolic action(s), and the synergistic effects that they might have, which could then lead to the development of new therapeutic agents.

As shown in Fig. 1, the ECS is extremely complex. Thus, to study the different EC pathways, identify their biological role(s), and find new therapeutical targets for various diseases, a holistic metabolomic approach is required. Given that ECs and related compounds are derivatives of fatty acids, lipidomic methods can be useful for studying the endocannabinoidome.

Understanding the endocannabinoid system

Given that the concentration of ECs varies substantially in many organs, tissues, and biofluids, particularly under inflammatory conditions [12], understanding the metabolism of ECs, especially the physiological role of the enzymatic systems involved in the synthesis and degradation of ECs, is key to supporting the development of new therapeutics [13]. Several recent reviews [7,14,15] have described the biosynthesis and fate of 2-AG and AEA. Although the levels of 2-AG and AEA can be modulated by the external supply of ECs or cannabinoids, inhibition of the enzymes involved in hydrolysis or modification of the CB receptors [12] requires a more holistic approach. However, only a couple of studies have focused on in-depth metabolomic or lipidomic analyses of the distribution of AEA and 2-AG, their precursors [*N*-acylphosphatidyl-ethanolamine (NAPE) 1,2-diacylglycerol (DAG), respectively), or by-products [e.g. arachidonic acid (AA), eicosanoids, prostaglandins, among others] [16,17].

In addition to the classic description of pathways in which ECs are involved, the transport of ECs across the cell membrane and the role of the ECS in the intercellular signaling that balances energy homeostasis have also been highlighted [18–20], in addition to the role of ECs in modulating the signaling pathways associated with altered mitochondrial function [21].

The ECS is involved in not only the CNS and crosstalk between the CNS and peripheral organs, but also the modulation of the physiology of various organs and systems, including the liver, cardiovascular system, musculoskeletal system, gastrointestinal tract, and immune system [1,19,22]. Recent studies that focused on the relation between the ECS and various diseases are detailed in Table 1.

Understanding the role of the ECS in several liver diseases has resulted in design of new drugs targeting the ECS [23]. In addition, the link between the gut microbiome and the host organism has been established by associations between gut bacteria and various diseases, and the interactions between the microbiome and the ECS in obesity, gut inflammation, and type 2 diabetes mellitus [24]. The role of ECs in the cardiovascular system is paradoxical because both positive and negative effects are observed, particularly in conditions of cardiovascular dysfunction [25,26]. Finally, the interactions between the ECS and signaling pathways involved in the control of cancer cell proliferation have also been highlighted [5,27].

The complexity of the signaling pathways in the CNS and the role of the ECS in healthy and diseased states are reflected by the number of clinical studies and the development of new drugs [28,29]. For example, a recent issue of *Biological Psychiatry* focused on cannabinoids and their role in psychotic disorders [30], with key reviews focusing on the signaling process [31,32] and ECS plasticity [33]. Thus, there is increasing interest in the role of the

TABLE 1

Summary of the relation between disease and the ECS

Disease and/or system affected	Analyzed tissue or fluid	Relevant ECs or receptors	Refs
Post-traumatic stress disorder	Peripheral blood	PEA (palmitoylethanolamide)	[34]
(cardiovascular and autoimmune diseases)	Hair	AEA, PEA, SEA, OEA	[35]
Spinal cord injury	Spinal cord	AEA, 2-AG	[56]
Psychiatric disorders	Immune function	AEA, 2-AG, modulators and metabolites	[57]
Vision	Retina, thalamus and cortical	AEA, 2-AG	[58][a]
	Retina and vision brain	2-AG and receptors	[59][a]
Diabetic retinopathy	Retina	CB1, CB2 and TRPV-1 receptor	[60][a]
Obesity and type 2 diabetes mellitus	Central and peripheral tissues	GPR18 receptor	[22]
Postprandial inflammation	Human plasma	NAGly, EPEA, POEA, DHEA, AEA, LEA, 2-AG, 2-LG, PEA, DEA, OEA, SEA	[61]
Osteoarthritis	Brain, spinal cord, and dorsal root ganglia	PEA, OEA, 2-AG, AraGly	[62]

[a] Review article.

ECS in neurodegenerative (PD, AD, among others) and psychological (schizophrenia, post-traumatic stress) disorders [34,35].

Testing new drugs

Studies to design new drugs to target the ECS focus on the use of natural and synthetic cannabinoids, and ECs, and below we describe some of the most recent publications.

Studies have shown that, during cancer treatment, activation of the ECS leads to apoptosis. The activation of autophagy as a consequence of treatment with cannabinoids has been reported for various tumor models. Thus, cannabinoids have been combined with anticancer drugs, such as temozolomide (TMZ), TNF-alpha-related apoptosis-inducing ligand (TRAIL), and gemcitabine (GEM), as well as radiation, to improve their antiproliferative effects [36].

Cannabinoids are also promising compounds for the treatment of neurological diseases. For example, THC, CBD, CBDV, and Sativex® (an oral-mucosal spray that contains the same concentration of THC and CBD) can be used: to delay the progression, and alleviate some of the cognitive symptoms of, AD; to treat the spasticity and pain associated with MS and HD; and as anticonvulsants in the treatment of a variety of epileptic syndromes, particularly untreatable pediatric epilepsies. There are also new therapeutic targets being reported, such as JZL184, a selective inhibitor of monoacylglycerol lipase (MAGL, the primary enzyme responsible for degrading 2-AG), which decreases the rate of neurodegeneration in PD, and the use of a dietary supplement of commercialized palmitoylethanolamide (PEA), such as Normast®, Pelvilen®, or Glialia®, to improve neurological functions and quality of life in patients with AD [2,37,38].

In terms of psychosis, high levels of serum AEA alleviate symptoms of, and have a protective role in, schizophrenia. Thus, new drug testing has focused on selective fatty acid amide hydrolase (FAAH) inhibitors, such as CBD [39]. Moreover, recent data suggest that CBD in combination with a CB_1 receptor neutral antagonist, such as AM4113, could not only augment the effects of standard antipsychotic drugs, but also target the metabolic, inflammatory, and stress-related components of schizophrenia [40].

For neuropathic pain, cannabinoids are used for diminishing chronic pain and spasticity; such cannabinoids include the synthetic cannabinoid ajulemic acid (CT3), CBD, THC, dronabinol (synthetic THC), levonantradol (CP 50,556-1, a synthetic analog of dronabinol), and nabilone (also a synthetic analog of dronabinol). Beneficial effects have been demonstrated with Sativex® in central pain in MS, brachial plexus avulsion, neuropathic pain after peripheral injury, and diabetic neuropathy. Trials have shown that using nabilone in patients with painful diabetic neuropathy reduced symptoms, and improved sleep disturbance and the quality of life. The latter has focused on the use of CB_2, FAAH, and MAGL to create more selective drugs that avoid the adverse effects of cannabimimetics [41,42].

During glaucoma therapy, THC and the synthetic CB_1 agonist WIN-55 212 are used for decreasing intraocular pressure (IOP). The topical application of THC and WIN-55 212 significantly reduces IOP without any psychotropic effects. Cannabinoids have a protective role in the retina, reducing oxidative stress signaling and preventing the neurodegeneration of retinal cells in age-related macular degeneration and diabetic retinopathy [43,44].

In infection-induced miscarriage, the levels of AEA and *N*-acylethanolamines (NAEs have an important role. Administration of lipopolysaccharide in mice increased the levels of AEA and NAEs in plasma and could be correlated with infection-induced miscarriages. To reduce such miscarriages, a feasible therapy could be the manipulation of CB_1 receptors and/or ligands [45].

Understanding the pathways and enzymes regulating the catabolism of AEA and 2-AG [such as cyclooxygenase (COX), lipoxygenase, αβ hydrolase, or members of the cytochrome P450 family] enables the study of many of the diseases mentioned above. However, more metabolomic or lipidomic studies are required to understand the conditions and factors that determine why AEA and 2-AG, as well as other overlooked ECs, are synthetized and degraded from one pathway to another. Such studies will help researchers determine the relations between pathways and the solution to new therapeutical targets for the treatment of these diseases.

Correlating cannabis plants with their therapeutic effectiveness

The idea of correlating cannabis plants with their therapeutic effectiveness is not new. Patients treated medically with cannabis have tried to find a variety that works optimally for treatment of their illness. As a result of limited knowledge and support from the medical community, they are usually guided by the popular understanding derived from recreational users. Thus, it is commonly accepted that therapeutic effects are mostly based on plant morphology. Preparations of *C. sativa* are described as uplifting and energetic, providing optimism and relieving pain for certain symptoms, whereas those from *Cannabis indica* are characterized as relaxing, relieving stress and body pain, and inducing feelings of calm and serenity [46].

However, apart from a few surveys of medical cannabis users discriminating the effects of *sativa* and *indica* [47,48], there is no scientific evidence to show that they have different physiological effects upon consumption. In a recent study, Elzinga *et al.* used scaled principal component analysis to investigate the analytical results for cannabinoids and terpenes in 494 cannabis flower samples from Californian patients taking medicinal marijuana, with a total of 35 different strains with at least eight different samples from each. In the comparison of samples characterized as either *sativa* or *indica*, the authors observed that most of the effects overlapped. Even when some *indica* samples were separated from the overlapping group because a different terpene content, the authors concluded that a new classification system is needed to further the medical utility of cannabis plants for use by patients, to enable them to communicate better with physicians and healthcare providers [49]. As reported by Sawler *et al.*, analysis of the genetic structure of 81 strains concluded that *sativa* and *indica* represent distinguishable pools of genetic diversity, but that narrow selective breeding has resulted in considerable admixture between the two [50].

Another drawback to correlating a *Cannabis* variety with specific health effects is the lack of plant standardization. Even though some companies are dispensing standardized plants, most users of medical cannabis consume nonstandardized plants. As an example, Elzinga *et al.* observed that the cannabinoid and terpene content is highly variable for Californian medical strains,

highlighting that the strain name cannot be used as indication of either potency or chemical composition [49].

As a result, experts in the field are encouraging the abandonment of the *sativa–indica* nomenclature and insisting that, to correlate strains with observed effects in real patients, cannabinoid and terpene profiles must be available for *Cannabis* in the medical and recreational markets [51]. Cannabinoids and the terpene β-caryophyllene are the only compounds in *Cannabis* able to bind CRs. However, terpenes can interact in synergy with cannabinoids and produce several physiological effects on their own [52]. Therefore, when plant material or extracts are used, a terpene profile should be available alongside the cannabinoid profile.

Terpenes are usually determined using gas chromatography coupled to flame ionization detector (GC-FID), by comparing the retention time with reference standards, mass spectra, and library data; by contrast, cannabinoid profiles are frequently obtained by high-performance liquid chromatography with diode-array detection (HPLC-DAD) [10]. However, to provide profiles that are as accurate as possible, new approaches have been recently developed. For example, an exhaustive cannabinoid fingerprinting method based on HPLC coupled to triple quadrupole and time-of-flight quadrupole mass analyzers was developed by Aizpurua-Olaizola *et al.* [53], and resolution of co-eluting compounds in the sesquiterpene and cannabinoid chromatographic region using 2D gas chromatography and multivariate curve resolution-alternating least squares was achieved by Omar *et al.* [54].

Thus, the combination of powerful analytical techniques and advanced chemometric tools provides an opportunity to assess the chemical composition of *Cannabis* holistically, which is a crucial step toward being able to correlate *Cannabis* plants with their therapeutic effectiveness.

Obtaining optimal cannabinoid mixtures for individual diseases

Even though most studies are based on the use of a single cannabinoid, it is well known that cannabinoids have synergistic effects when interacting each other, which could be beneficial for the treatment of several diseases. Recent work by Gallily *et al.* clearly supports this idea [55]. These authors discovered that, in mice, unlike the bell-shaped dose–response curve of pure CBD, the anti-inflammatory and antinociceptive activities of standardized CBD-enriched plant extracts increased with dose, making whole-plant extracts superior to pure CBD for the treatment of inflammatory conditions. However, most of the phytocannabinoids remain unexplored and, as a result, there is a lack of understanding of how minor compounds can alter the effects of major compounds.

As shown in Fig. 1, there are many CRs that can be activated, inhibited, or modulated by various ECs and cannabinoids. Moreover, it is well known that CRs can interact in diverse ways with different cannabinoids and at different physiological concentrations. These CRs are relevant to diverse bodily functions and, thus, to different diseases. However, as previously mentioned, most cannabinoids are unexplored and many of the interactions studied between major cannabinoids and CRs have been shown to occur *in vitro* but their relevance *in vivo* is yet to be clarified [11].

Thus, a useful next step could be the creation of a cannabinoid–receptor interaction matrix (CRIM). There are at least 113 cannabinoids in cannabis [10], most of which are unexplored, but which could offer thousands of solutions to create optimal cannabinoid mixtures to achieve a particular effect for the treatment of each specific disease. These unexplored cannabinoids could be also a source of new synthetic cannabinoids. Finally, the addition of terpenes should also be considered to strengthen the desired effects.

To create the CRIM, the extraction and purification of minor cannabinoids before use in biomedical assays, and exhaustive meta-analyses of reported values will be necessary. Once the optimal combinations and appropriate routes of application for each disease have been identified, the final step will be to test them in clinical trials. This approach, along with assessing the chemical composition of plants holistically, would also make it possible to correlate standardized plant compounds with their therapeutic effects.

Concluding remarks

There is an increasing body of knowledge surrounding the ECS and its role in several diseases. As a consequence, new drugs based on cannabinoids and cannabinoid-like compounds have been tested in recent years. Although the results have been promising, a better understanding of pathways, interactions of cannabinoids with CRs, and synergistic effects both between cannabinoids and between cannabinoids and terpenes could result in improved and more specific drugs. Thus, we suggest that the use of metabolomic approaches to study key pathways, and the creation of a CRIM are important steps toward the next generation of cannabinoid-based drugs.

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