Pharmacological and Toxicological Effects of Synthetic Cannabinoids and Their Metabolites

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Abstract Commercial preparations containing synthetic cannabinoids (SCBs) are rapidly emerging as drugs of abuse. Although often assumed to be "safe" and "legal" alternatives to cannabis, reports indicate that SCBs induce toxicity not often associated with the primary psychoactive component of marijuana, Δ9-tetrahydrocannabinol (Δ^9-THC). This chapter will summarize the evidence that use of SCBs poses greater health risks relative to marijuana and suggest that distinct pharmacological properties and metabolism of SCBs relative to Δ^9-THC may contribute to this increased toxicity. Studies reviewed will indicate that in contrast to partial agonist properties of Δ^9-THC typically observed in vitro, SCBs act as full CB1 and CB2 receptor agonists both in cellular assays and animal studies. Furthermore, unlike Δ^9-THC metabolism, several SCB metabolites retain high affinity for and exhibit a range of intrinsic activities at CB1 and CB2 receptors. Finally, the potential for SCBs to cause adverse drug–drug interactions with other drugs of abuse, as well as with common therapeutic agents, will be discussed. Collectively, the evidence provided in this chapter indicates that SCBs should not be considered safe and legal alternatives to marijuana. Instead, the enhanced toxicity of SCBs relative to marijuana, perhaps resulting from the combined actions of a complex mixture of different SCBs present and their active metabolites that retain high affinity for CB1 and CB2 receptors, highlights the inherent danger that may accompany use of these substances.

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Contents

1 Introduction

Synthetic cannabinoids (SCBs) have become popular recreational drugs among young adults in the USA. Use of these substances first emerged in Europe, where they were marketed as *Spice*, then quickly spread throughout the USA, where they were marketed as *K2* [1]. SCBs are sometimes marketed for commercial distribution in the form of capsules, tablets, and powders but are most commonly laced onto herbal mixtures (e.g., potpourri or incense) to be smoked (as reviewed by Tai and Fantegrossi [2]). Commercial SCB products are widely available on the internet and, despite regulatory efforts to curtail their availability, remain accessible at "brick and mortar" establishments such as head shops and convenience stores. Their widespread distribution can be attributed to clever marketing tactics, which usually involve colorful packaging and mislabeling designed to portray a harmless herbal blend which is "not intended for human consumption." These deceptive marketing tactics were adopted to circumvent legal ramifications for selling drugs of abuse, but it is unlikely that criminal proceedings stemming from the sale of prohibited substances would be impeded by arguments about product labels. Importantly, the marketing of these products appears to be specifically designed to give users the false assumption that these drugs are harmless, "natural," legal alternatives to cannabis.

Examining the pharmacological similarities between SCBs and Δ9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive constituent in marijuana, has been a topic of great interest among scientists and lawmakers. In this regard, SCBs have been reported to exhibit higher binding affinity at both CB1 and CB2 cannabinoid receptor (CBR) subtypes, and also to display varying intrinsic activity relative to Δ^9-THC, both in cellular assays and animal studies [3–7]. Unlike Δ^9-THC, which consistently exhibits partial agonist efficacy in vitro [4, 5], SCBs are fully efficacious at both CBRs across a range of in vitro and in vivo assays [4, 5, 8]. In addition, metabolites of SCBs often retain higher CBR affinity than Δ^9-THC and may elicit pharmacological and toxicological effects distinct from those induced by Δ^9-THC. These active metabolites could potentially explain the increased morbidity and mortality associated with SCB exposure, as compared to what is typically

seen with marijuana. The remainder of this chapter will discuss the pharmacological and toxicological effects related to the metabolism of SCBs with a particular emphasis on their active metabolites and show that these substances are not safe, have a greater toxicological profile than has been reported with marijuana, and should not be considered a legal alternative to cannabis.

2 Synthetic Cannabinoid Metabolism

Metabolism of xenobiotics occurs through several biotransformation pathways which are conserved across species and quite old from an evolutionary perspective. The goal of drug metabolism is to detoxify potentially harmful compounds, removing them from the circulation and ultimately excreting them from the body altogether. The liver plays a major role in this detoxification process. In some cases, metabolism may activate inert compounds (the concept of a "pro-drug") or produce metabolic intermediates which may themselves induce toxicity. In most cases, oxidative metabolism of xenobiotics first occurs via the hepatic cytochrome P450 (CYP) enzyme system at which point the metabolites are conjugated with a sugar moiety, glucuronic acid, by a class of enzymes called UDP-glucuronosyltransferases (UGTs). The resulting metabolites are then soluble enough to be removed from the body.

The metabolism of Δ^9-THC has been a reference standard for understanding cannabinoid pharmacokinetics [9]. Metabolism of Δ^9-THC by human hepatic microsomes initially occurs via oxidation by CYP subtypes 2C9 and 3A4 [10]. In brief, hydroxylation of Δ^9-THC by CPY2C9 produces 11-hydroxy-Δ^9-THC, which is the only psychoactive metabolite of Δ^9-THC [11, 12]. Further oxidation of the remaining hydroxyl groups of Δ^9-THC produces carboxylic acids at several positions along the alkyl side chain, and these metabolites are devoid of biological effects. Further oxidation of the active metabolite 11-hydroxy-Δ^9-THC abolishes pharmacological activity and leads to the production of 11-nor-9-carboxy-Δ^9-THC, which is then conjugated at the carboxyl position to form *O*-ester glucuronide, the major metabolite excreted in human urine [13].

In the early 2000s, initial reports of SCB metabolism emerged with a focus on in vitro metabolism of CBR ligands (11R)-2-methyl-11-[(morpholin-4-yl)methyl]-3-(naphthalene-1-carbonyl)-9-oxa-1-azatricyclo[6.3.1.$0^{4,12}$]dodeca-2,4(12),5,7-tetraene (WIN-55,212-2) [14], 1-[2-(morpholin-4-yl)ethyl]-2-methyl-3-(4-methoxybenzoyl)-6-iodoindole (AM-630) [15], and (2-methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone (JWH-015) [16]. The subsequent recreational use of SCBs shifted the focus more towards in vivo metabolism of the commonly abused CBR ligand naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018) and its analogs. Metabolites of JWH-018 were first identified in urine specimens obtained from three people who had smoked a commercial product containing this compound [17]. Predominant phase I metabolites are formed by oxidation of the indole ring or the N-alkyl chain to form mono-hydroxylated compounds. The phase I JWH-018 metabolites are excreted in urine almost exclusively in the form of phase II glucuronide conjugates, as

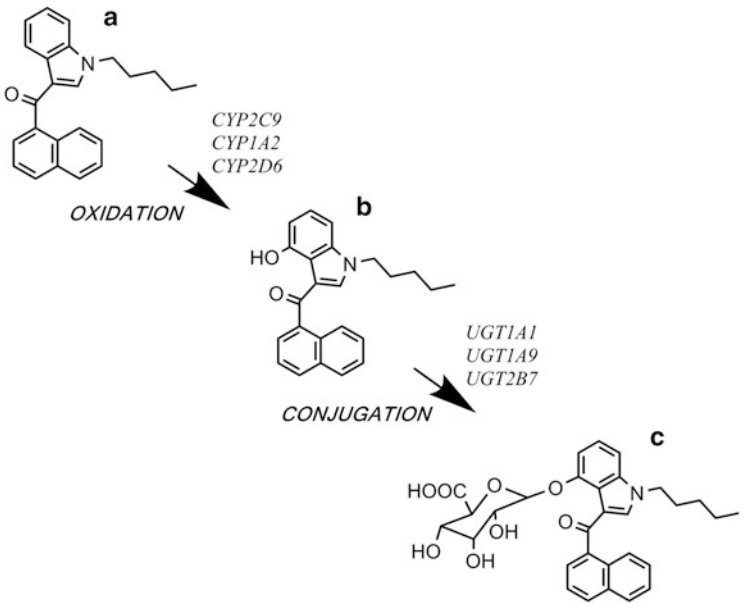

Fig. 1 Metabolism of the synthetic cannabinoid (SCB) JWH-018. The parent compound JWH-018 (**a**) undergoes phase I oxidation by cytochrome P450 (CYP) enzymes to form the bioactive JWH-018 4-hydroxyindole metabolite (**b**) [5]. Phase II conjugation by UDP-glucuronosyltransferases (UGTs) forms the corresponding glucuronide conjugate (**c**) [18]. Specific CYP enzymes and UGTs responsible for these biotransformation reactions are noted

determined by gas- and liquid-chromatography mass spectrometry (GC-MS and LC-MS/MS). Figure 1 depicts the metabolism of JWH-018 to its 4-hydroxyindole metabolite and corresponding glucuronide conjugate. Subsequent investigations demonstrated the formation of phase I mono-hydroxylated metabolites and phase II glucuronides in human hepatic microsomes incubated with JWH-018 [19], urine specimens obtained from individual users of *Spice*/*K2* products, and from rats administered JWH-018 [20, 21], naphthalen-1-yl-(1-butylindol-3-yl)methanone (JWH-073) [22], or 2-(2-methoxyphenyl)-1-(1-pentylindol-3-yl)ethanone (JWH-250) [20]. Collectively, these studies confirmed that the primary urinary metabolites of these SCBs are excreted in the form of a single hydroxylation and subsequent glucuronidation.

It was not until 2011 that reference standards for identifying specific JWH-018 and JWH-073 metabolites were developed allowing for both in vivo and in vitro quantitative measurements by LC-MS [18, 23, 24]. Human urine specimens were obtained from individuals who ingested JWH-018 or a mixture of JWH-018 and JWH-073. Analysis determined that the metabolites found in the urine were excreted in high concentrations and primarily in the form of glucuronic acid conjugates [18]. Several additional laboratories have replicated with these findings [20, 25–30].

It is well accepted that CYPs are involved in the biotransformation of SCBs in humans as recognized by the formation of hydroxylated metabolites [14, 17–19, 24, 29]. In vitro metabolism studies have determined that SCBs JWH-018 and its fluorinated analogue 1-[(5-fluoropentyl)-1H-indol-3-yl]-(naphthalen-1-yl)methanone (AM-2201) are metabolized via oxidation by hepatic CYP subtypes 2C9 and 1A2 [23]. Outside the liver, CYPs are ubiquitously expressed in the body in a tissue-specific manner. CYP2C9 is also highly expressed in the intestine [31], so it is likely that intestinal CYP2C9 is involved in the metabolism of SCBs when ingested orally. CYP1A2 is highly expressed in the lung and is likely responsible for the metabolism of smoked SCBs [32]. There is evidence of the involvement of CYP2D6 in the metabolism of JWH-018 and AM-2201 in the brain especially in brain regions that have a high expression of CB1Rs, including the cortex, hippocampus, and cerebellum. It is likely that CYP2D6 is involved in the management of brain concentrations of these SCBs and their active metabolites, more so than in the liver.

Urine specimens obtained from individuals who ingested SCBs contain high concentrations of glucuronide metabolites [17–19, 24], coupled with no traces in serum [33, 34], suggesting that glucuronic acid conjugation plays a key role in the excretion of these drugs in urine. In the liver, JWH-018 and JWH-073 metabolites are formed by major UGT isoforms UGT1A1, UGT1A9, and UGT2B7 [18] (see Fig. 1). In addition, extrahepatic UGT isoforms involved in metabolism include UGT1A7 expressed in lung, UGT1A3 and UGT2B7 expressed in brain (as well as liver), and UGT1A10. Importantly, human UGT1A3 and UGT2B7 are predominant isoforms responsible for generating the major metabolites of JWH-018 and JWH-073 found in urine [35]. Since UGT1A3 and UGT2B7 are also expressed in the brain, these UGT isoforms may play a role in regulating brain concentrations of SCBs and their active metabolites at the CB1R, similar to CYP2D6 (as previously discussed).

3 Synthetic Cannabinoid Cellular Signaling

The pharmacological profiling of Δ^9-THC has led to the characterization of metabolites produced by SCBs JWH-018 and JWH-073 [36]. Like the phytocannabinoid Δ^9-THC, both JWH-018 and JWH-073 have high affinity for the CB1 and CB2Rs [3–7]. Chemical structures for JWH-073 many of the related SBCs discussed in this chapter are shown in Fig. 2. Emerging SCBs found in commercial preparations also have high affinity for the CB1 and CB2Rs, including 2-[(1S,3R)-3-hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol (CP-47,497) [37], (1-pentylindol-3-yl)naphthalen-1-ylmethane (JWH-175) [38], 1-([(1E)-3-pentylinden-1-ylidine]methyl)naphthalene (JWH-176) [38], (1-pentylindol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone (UR-144) [39], naphthalen-1-yl-(1-pentylpyrrol-3-yl)methanone (JWH-030) [40], 2-(2-methoxyphenyl)-1-(1-pentylindol-3-yl)ethanone (JWH-250) [41], N-(1-adamantyl)-1-pentylindazole-3-carboxamide (APINACA, or AKB48) [42], and 1-[(N-methylpiperidin-2-yl)methyl]-3-(adamant-1-oyl)indole (AM-1248) [43]. Furthermore, most commercial SCB products display high potency and efficacy as CB1R

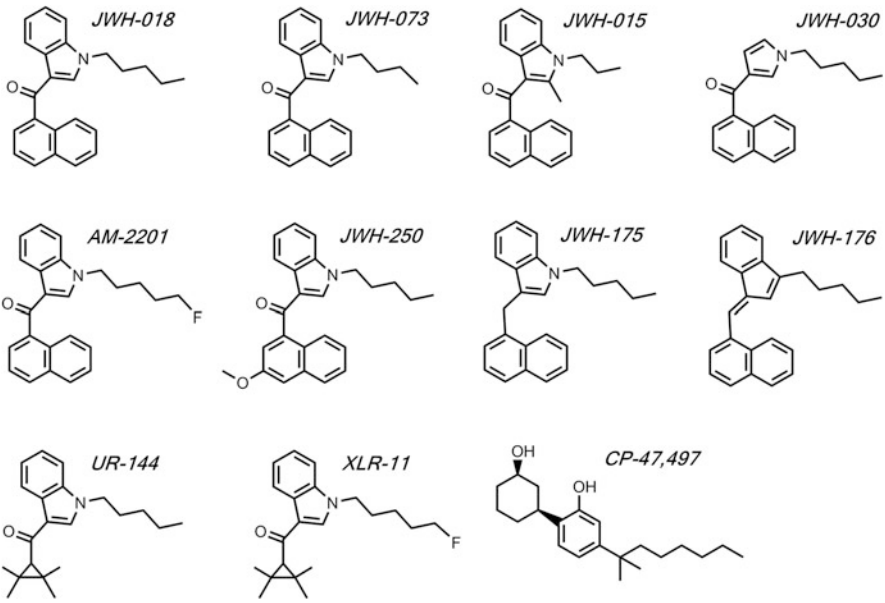

Fig. 2 Chemical structures of SCBs discussed in this chapter. Abbreviations are as follows: *JWH-018* naphthalen-1-yl-(1-pentylindol-3-yl)methanone, *JWH-073* naphthalen-1-yl-(1-butylindol-3-yl)methanone, *JWH-015* (2-methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone, *JWH-030* naphthalen-1-yl-(1-pentylpyrrol-3-yl)methanone, *AM-2201* 1-[(5-fluoropentyl)-1H-indol-3-yl]-(naphthalen-1-yl)methanone, *JWH-250* 2-(2-methoxyphenyl)-1-(1-pentylindol-3-yl)ethanone, *JWH-175* (1-pentylindol-3-yl)naphthalen-1-ylmethane, *JWH-176* 1-([(1E)-3-pentylinden-1-ylidine]methyl)naphthalene, *UR-144* (1-pentylindol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone, *XLR-11* (1-(5-fluoropentyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone, *CP-47,497* (2-[(1S,3R)-3-hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol

agonists both in vitro and in vivo, including JWH-018 [5, 8], JWH-073 [4, 44], AM-1248 [39], CP-55,940 [40], WIN-55,512-2 [40], and (6aR,10aR)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6H,6aH,7H,10H,10aH-benzo[c]isochromen-1-ol (HU-210) [45].

The abuse liability of SCB products may be attributed to the presence of high affinity and fully efficacious CB1 agonists in these commercial products [46]. For instance, the in vitro efficacy of Δ^9-THC is partial relative to JWH-018 and JWH-073, which are fully efficacious [4, 8, 46]. Importantly, not all in vitro or ex vivo assessments can identify differences in efficacy between THC and SCBs. An interesting study by Hoffman et al. [47] demonstrated similar efficacy for SCBs and THC in an electrophysiological assay reflecting inhibition of transmitter release. Nevertheless, the often demonstrated low in vitro efficacy of Δ^9-THC does not necessarily translate to partial agonism in vivo, and Δ^9-THC often displays in vivo efficacy comparable to fully efficacious agonists [48]. In addition, abrupt discontinuation of chronic marijuana use or Δ^9-THC administration produces a withdrawal syndrome in humans and rodents that is accompanied by a region-specific downregulation and desensitization

of CB1Rs in the brain [49–51]. Thus, it is possible that commercial SCB products that contain high-efficacy agonists may intensify the adverse effects related to tolerance, dependence, and withdrawal of SCB abuse relative to Δ^9-THC. For instance, the commercial SCB product "Spice Gold" produced a withdrawal phenomenon and dependence syndrome in humans that transpired after abrupt discontinuation of use in the form of drug craving, elevated blood pressure, nausea, tremor, profuse sweating, and nightmares [52]. The active constituents of this product were not forensically determined in the product itself or in fluids or tissue from the case subject, but contemporaneous laboratory studies determined that a mixture of the SCBs JWH-018 and CP-47,497 was present in this commercial smoking blend at the time and in the geographic area where the aforementioned case occurred [52].

Metabolism of Δ^9-THC produces a single active metabolite (11-hydroxy-Δ^9-THC) that exhibits reduced CB1R affinity compared with the parent compound [53]. On the other hand, metabolism of commercial SCBs including JWH-018, JWH-073, and AM-2201 produces numerous major mono-hydroxylated metabolites that retain nanomolar binding affinity for CB1Rs [4, 5, 23] (see Fig. 1), unlike their carboxylated metabolites, which do not bind to nor activate CB1Rs.

In addition to retaining high CB1R affinity, in vitro functional assays (G-protein activation) demonstrate that the major mono-hydroxylated metabolites of JWH-018, JWH-073, and AM-2201 exhibit partial to full efficacy at the CB1Rs similar to fully efficacious CP-55,940 [4, 5, 23]. Of further importance, the in vivo cannabimimetic effects of JWH-018 and JWH-073 mono-hydroxylated metabolites elicited profound hypothermic and locomotor depressant effects in mice [4]. These effects are attenuated by CB1R antagonist/inverse agonist 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-(1-piperidyl)pyrazole-3-carboxamide (AM-251), suggesting that these metabolites are mediating their effects through the CB1R, similar to the parent ligands. Moreover, the effects elicited by these metabolites may be associated with adverse effects of SCB use. For instance, it has been shown that several mono-hydroxylated metabolites of JWH-018, JWH-073, and AM-2201 retain high CB1R affinity and activity and may display additive or synergistic interactions with other SCBs [54]. Additional contributing factors to adverse effects of SCB use can be: (1) drug effects of non-cannabinoid-like ligands found in commercial SCB products, (2) variations in batch-to-batch preparations with differences in the concentration and content found in commercial SCB products, and (3) an exacerbation of SCB adverse effects in drug users with preexisting conditions [36, 55–58].

Although evidence suggests that mono-hydroxylated metabolites of JWH-018, JWH-073, and AM-2201 are active at CB1Rs in both in vitro and in vivo assays [54], it is also possible for some oxidized metabolites of SCBs to function as antagonists at CB1Rs. Despite the fact that the 7-hydroxyindole derivative of JWH-073 has not been detected in human urine, it has been shown to bind to CB1Rs with nanomolar affinity without eliciting any G-protein activation at concentrations that are pharmacologically relevant, up to 10 μM [4]. Furthermore, Schild analysis has shown that the 7-hydroxyindole derivative of JWH-073 competitively antagonizes G-protein activation in vitro. In mice, hypothermia induced by JWH-018 was attenuated by pretreatment with this oxidized derivative of JWH-073. Alternatively,

JWH-018 induced analgesia, catalepsy, and locomotor activity were not altered by this metabolite. Overall, these in vitro and in vivo findings along with the lack of the 7-hydroxyindole derivative of JWH-073 detected in human urine suggest that this oxidized derivative of JWH-073 may not be formed in humans or readily cross the blood–brain barrier.

In addition to a human oxidation product of JWH-018 acting as a CB1R antagonist, it has also been shown that a major human glucuronidated metabolite of JWH-018 (5-hydroxypentyl-β-D-glucuronide) retains significant affinity for CB1Rs and displays CB1R antagonism in vitro [59]. Interestingly, this study also showed that a major structurally similar glucuronidated metabolite of THC (11-nor-9-carboxy-THC-β-D-glucuronide) lacked CB1R affinity and activity. Collectively, these findings demonstrate that both hydroxylated and glucuronidated metabolites of SCBs may retain significant CB1R affinity in the absence of intrinsic activity, which suggest that some SCB metabolites can produce physiologically relevant antagonism of effects of CB1Rs.

When discussing the pharmacological and toxicological effects of SCBs, the primary focus has been geared towards understanding CB1R-induced responses. Many SCBs not only have high affinity and significant intrinsic activity at the CB1R, they very often also have comparable binding and functional activity at the second major CBR subtype, CB2R [3, 6]. CB1Rs are primarily located within the CNS, while CB2Rs are most abundantly located on immune cells in peripheral regions [60] and are associated with immune functions, inflammation, and bone formation [61]. More recent studies have demonstrated that activation of low numbers of CB2R in the CNS can modulate the abuse-related properties of alcohol [62], nicotine [63], and cocaine [64]. In addition, mono-hydroxylated metabolites of JWH-018 and JWH-073 have also been shown to retain high CB2R affinity and efficacy [7].

Interestingly, findings have implicated the involvement of endocannabinoid signaling in the modulation of the serotonin system (as reviewed by Haj-Dahmane and Shen [65]. For instance, chronic activation of CB2Rs has been shown to produce an upregulation of 5-HT_{2A} receptors in the prefrontal cortex of mice [66, 67]. CNS abnormalities in 5-HT_{2A} function can lead to mental disorders, including anxiety [68] and psychosis [69]. Furthermore, 5-HT_{2A} receptor signaling is a major site of action for hallucinogenic drugs [70]. Common adverse effects associated with SCB use are not often observed with Δ^9-THC, such as anxiety and psychosis [71]. It is possible to speculate that SCB- or SCB metabolite-induced upregulation of 5-HT_{2A} receptors, mediated via CB2R activation, might contribute to anxiety and psychosis that are observed after exposure to SCBs found in commercial abuse-ready preparations. In this regard, a case study reported on four patients hospitalized for psychosis who smoked a product containing the SCB AM-2201 while in the clinic. The authors described the appearance of new psychotic symptoms, and a marked worsening of mood and anxiety symptoms in four patients, and noted that even though they all ingested the same drug, the clinical picture differed markedly among the individual patients [72], perhaps implicating individual metabolism of AM-2201 in the diversity of effects observed. Thus, it is clearly important for future

studies to explore both CB1R and CB2R signaling as it relates to the pharmacological and toxicological effects of SCBs and its metabolites. Furthermore, future studies should consider the capacity of these drugs to modulate the expression and function of other, non-CBR systems.

4 Synthetic Cannabinoid Drug–Drug Interactions

Commercial SCB preparations often contain multiple drugs in combination, and the concentrations of these specific SCBs vary widely from product to product, or even within a product from batch to batch [58, 59]. Therefore, it is possible for drug–drug interactions to exist both within and between these diverse mixtures of SCBs, which may contribute to abuse-related and adverse effects associated with the use of these drugs. As described above, mouse studies have shown that coadministration of JWH-018 and JWH-073 produced additive, synergistic, or antagonistic interactions compared to administration of either drug alone, depending on the specific endpoint examined and the drug dose ratio employed [54]. Evidence of synergistic effects with these two SCBs was demonstrated both in vivo and in vitro, with mouse assays of Δ^9-THC discrimination and analgesia, and with displacement of radioligand binding from CB1Rs in a cellular model. In addition to synergism, SCB blended mixtures can influence the relative potency of both their subjective and adverse effects. Furthermore, polysubstance abuse may lead to unpredictable effects of SCBs that may contribute to even greater abuse-related and adverse effects. Future studies are needed to understand the drug–drug interactions among SCBs and co-exposure to other drugs of abuse.

Given their shared metabolism via P450 isoforms, combined use of SCBs with various prescription medications could also potentially result in adverse drug–drug interactions. Commonly prescribed drugs such as valproic acid (an anticonvulsant and mood stabilizer) and sertraline (an antidepressant) potently inhibit CYP2C9, while drugs such as ciprofloxacin (an antibiotic) and fluvoxamine (an antidepressant) strongly inhibit CYP1A2. Additionally, CYP2C9 is a major polymorphic enzyme [31] and is responsible for the metabolism of a number of clinically important drugs such as warfarin (a blood thinner), phenytoin (an anticonvulsant), tolbutamide (an antidiabetic agent), losartan (an antihypertensive), and ibuprofen (a nonsteroidal anti-inflammatory drug). Over five allelic variants of CYP2D6 have been identified, including two "loss of function" variants (CYP2C9*4 and CYP2C9*5) [73]. Similarly, CYP1A2 is responsible for the metabolism of numerous psychiatric medications including antipsychotics (olanzapine, clozapine, haloperidol, and thioridazine), antidepressants (imipramine, clomipramine, and fluvoxamine), and cholinesterase inhibitors used in the treatment of Alzheimer's disease (tacrine) [74], but this enzyme is well conserved without common functional polymorphisms [75]. Because SCBs are also substrates for these P450 isoforms, the possibility of drug–drug interactions is a serious consideration with the use of SCBs.

5 Conclusions

Commercial SCB products are not safe alternatives to cannabis and pose significant threats to public health. It is anticipated that morbidity and mortality rates will continue to increase in correlation to SCB exposure. Recent reports have demonstrated that SCBs present a pharmacological and toxicological profile that is distinct from Δ^9-THC found in marijuana. For instance, SCBs primarily act as full CB1 and CB2R agonists both in vitro and in vivo, while Δ^9-THC is a weak partial agonist. Furthermore, several SCB metabolites bind with high affinity at CB1 and CB2Rs, while displaying a range in intrinsic activities from neutral antagonists to partial agonists to full agonists, both in cellular assays and animal studies. These findings illustrate that commercial SCBs products are not safe and should not be considered an alternate form of marijuana. Rather, they produce greater toxicity relative to marijuana, which could be attributed to the combined actions of the varying SCBs or their metabolites present in these products. Still, these findings present the supporting evidence that the pharmacological and toxicological properties of SCBs pose a severe health risk.

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