Combination Chemistry: Structure–Activity Relationships of Novel Psychoactive Cannabinoids

Jenny L. Wiley, Julie A. Marusich, and Brian F. Thomas

Abstract Originally developed as research tools for use in structure–activity relationship studies, synthetic cannabinoids contributed to significant scientific advances in the cannabinoid field. Unfortunately, a subset of these compounds was diverted for recreational use beginning in the early 2000s. As these compounds were banned, they were replaced with additional synthetic cannabinoids with increasingly diverse chemical structures. This chapter focuses on integration of recent results with those covered in previous reviews. Whereas most of the early compounds were derived from the prototypic naphthoylindole JWH-018, currently popular synthetic cannabinoids include tetramethylcyclopropyl ketones and indazole-derived cannabinoids (e.g., AB-PINACA, AB-CHMINACA). Despite their structural differences, psychoactive synthetic cannabinoids bind with high affinity to CB_1 receptors in the brain and, when tested, have been shown to activate these receptors and to produce a characteristic profile of effects, including suppression of locomotor activity, antinociception, hypothermia, and catalepsy, as well as Δ^9-tetrahydrocannabinol (THC)-like discriminative stimulus effects in mice. When they have been tested, synthetic cannabinoids are often found to be more efficacious at activation of the CB_1 receptor and more potent in vivo. Further, their chemical alteration by thermolysis during use and their uncertain stability and purity may result in exposure to degradants that differ from the parent compound contained in the original product. Consequently, while their intoxicant effects may be similar to those of THC, use of synthetic cannabinoids may be accompanied by unpredicted, and sometimes harmful, effects.

Keywords Cannabinoids • Indoles • JWH-018 • Receptor binding • Synthetic cannabinoids • THC

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Contents

1 Introduction

Synthetic cannabinoids are chemicals that interact with the endogenous system through which the psychoactive components of the cannabis plant act. Although these compounds were originally developed as tools for probing receptors and/or as part of early phase drug discovery efforts, reports from drug abuse monitoring sites beginning in the early 2000s suggested that some of these research chemicals were being diverted for recreational use. These compounds, contained in products labeled "Spice" or "herbal incense," shared the cannabimimetic subjective effects of Δ^9-tetrahydrocannabinol (THC), the primary psychoactive constituent of the cannabis plant. However, because they differed structurally from THC and its analogs, synthetic cannabinoids were legal when they were first marketed as recreational drugs. Since then, drug enforcement agencies worldwide have struggled to develop strategies to manage the continuous influx of novel synthetic cannabinoids that have increased in structural diversity as older compounds have been banned. This chapter presents an overview of the context in which synthetic cannabinoids were discovered followed by a more in-depth look at their pharmacology.

Determination of the strength of relationships between the chemical structures of compounds and their activity is fundamental to the science of pharmacology as well as to the process of drug discovery and development. Orderly structure-activity relationships (SAR) are a cardinal sign of receptor activation or blockade, processes through which many drug treatments for disorders of the central nervous system (CNS) work. Synthetic cannabinoids were originally designed and synthesized for use in SAR studies in academic and pharmaceutical laboratories. Although computational chemistry and other technological advances in recent years have led to innovative approaches to drug discovery, assessment of SAR was considered "state-of-the-science" in investigation of receptor mechanisms in the 1980s and early 1990s when the story of non-plant-based synthetic cannabinoids began. Cannabinoid receptors (CB_1 and CB_2) also were identified and cloned during this time of intensive SAR evaluation [1–3]. Furthermore, synthesis of a radiolabeled

synthetic cannabinoid, [^{3}H]CP55,940, played an integral part in discovery of this receptor system [4].

The endocannabinoid system is comprised of CB_1 and CB_2 receptors, along with their endogenous ligands [e.g., anandamide and 2-arachidonoylglycerol (2-AG)] and synthetic and metabolic enzymes for these ligands (reviewed in [5]). Both cannabinoid receptors are G-protein coupled receptors, with CB_1 receptors located widely throughout the brain and CB_2 receptors found primarily in the periphery [6–8]. THC is a partial agonist at both receptor sub-types [9, 10], but produces its cannabimimetic psychoactive effects via activation of CB_1 receptors in the CNS [11], as do synthetic cannabinoids [12, 13]. While medical and legal problems associated with the manufacture and use of synthetic cannabinoids are causes for concern, these compounds were created within a research context, were not meant for human use without further development, and contributed to significant scientific advances in the cannabinoid field. For example, these pharmacological tools aided researchers in discovery of CB_1 and CB_2 receptors [1], delineation of separate functions mediated by CB_1 and CB_2 receptors [14, 15], determination of CB_1 receptor mediation of cannabis intoxication [11], and demonstration of possible roles that the endocannabinoid system may play in physiology and pathology [5].

For many years, the focus was on synthesis of compounds that directly activated or blocked cannabinoid receptors; however, separation of psychoactive and therapeutic effects proved problematic for compounds that activated CB_1 receptors directly. In addition, the considerable homology between CB_1 and CB_2 receptors presented difficulties in designing CB_2-selective compounds that did not activate CB_1 receptors. Hence, many of the earlier compounds bind to and activate both cannabinoid receptors. More recently, the scope of cannabinoid synthesis has broadened to include compounds that inhibit endocannabinoid metabolic enzymes (e.g., inhibitors of fatty acid amide hydrolase and monoacylglycerol lipase for anandamide and 2-AG, respectively) [16–18]. These new compounds, as well as selective CB_2 receptor agonists, offer promising leads for development of therapeutics to treat the many disorders or conditions that may be related directly or indirectly to dysfunction of the endocannabinoid system, including pain, neurodegeneration, substance abuse, obesity, and psychiatric disorders [19–22].

2 Diversion and Development of an Illicit Industry

Diversion of synthetic cannabinoids from their use in research was first recognized by drug enforcement agencies across the world during the early 2000s and has increased in scope since then. The concomitant rise of the worldwide web and its public availability facilitated the spread of awareness of these chemicals [23], including greater access to patent literature and to scientific papers on synthetic methods. Further, the web provided opportunities for direct marketing to consumers and organization of forums of like-minded drug users to spread information on the latest compounds. The synergistic effects of these developments arguably led to the

rapid proliferation of synthetic cannabinoid use. Currently, the primary location for bulk production of the compounds is believed to be China [24].

Once synthesized, the compounds are shipped to product manufacturers who spray the cannabinoid compound(s) on plant material (e.g., marshmallow leaf) and package it for individual sale. Even though the packets often are labeled as "not for human consumption," users use the substance via the same methods employed with cannabis, such as rolling the material into smokeable joints or placing it in pipes or other devices for smoking. Two notable issues arise as a direct result of this process. The first is that spraying often leads to uneven distribution of the chemical across the sample contained in the package, creating the possibility of "hot spots" containing enhanced concentrations of active chemicals. The second is that the combustion involved in smoking the plant material can conceivably alter the composition of the chemical it contains, resulting in exposure to different chemicals [25]. These problems have not been ameliorated by the recent switch by some users to e-liquids that contain synthetic cannabinoids. Given the low solubility of cannabinoids in the e-liquid vehicle, crystallization or precipitation may occur and use of atomizers or vaporizers marketed for nicotine still involve intense heating of the chemical.

Metabolism of the synthetic cannabinoids may also result in creation of additional chemicals that may modulate and/or extend the duration of its effects [26–28].

3 Receptor Affinity and Efficacy

Like phytocannabinoid agonists, synthetic cannabinoids exhibit structurally specific receptor recognition and can affect the activation state of the receptor in a variety of signal transduction pathways. Assessment of cannabinoid receptor recognition is typically affected by measurement of the strength with which the synthetic cannabinoid displaces a radiolabeled ligand that binds to the receptor (e.g., [^{3}H]CP55,940) and is expressed as affinity (k_i), with lower numbers indicative of higher affinity. The ability of synthetic cannabinoids to alter the activation state of cannabinoid receptors (i.e., efficacy) has been measured through examination of their effects on signaling pathways (e.g., GTPγS, beta arrestin, and calcium influx). In general, synthetic cannabinoids tend to have greater affinities for the CB_1 receptor than does THC, which often correlates with their greater potencies in vivo. To the extent that they have been evaluated, synthetic cannabinoids also show greater efficacy for the CB_1 receptor than the partial agonist THC [13, 29, 30]. The acute in vivo correlates of greater efficacy are not fully understood, although some research suggests that efficacy differences may have implications for development of tolerance and cross-tolerance following repeated administration [31].

THC and most of the abused synthetic cannabinoids bind to and activate both CB_1 and CB_2 receptors, with variable degrees of selectivity for one or the other

receptor. Cannabimimetic psychoactivity of the compounds, and their consequent abuse liability, is most closely associated with their high CB_1 receptor affinities [11]; hence, this section maintains a primary focus on CB_1 receptor SAR, with minimal attention to CB_2 and noncannabinoid receptors.

3.1 CB_1 Receptor

Until recently, the most prevalent synthetic cannabinoids identified in spice or herbal incense products were classified into seven structural groups, as depicted in Fig. 1: naphthoylindoles (e.g., JWH-018, JWH-073, and AM-2201), naphthylmethylindoles (JWH-185), naphthoylpyrroles (JWH-030), naphthylmethylindenes (JWH-176), phenylacetylindoles (JWH-250, RCS-4), cyclohexylphenols (CP47,497), and tetrahydrocannabinols (THC, HU-210). Previous publications have reviewed the in vitro and in vivo pharmacology of indole- and pyrrole-derived cannabinoids [32, 33]. Systematic legal restrictions placed on these cannabinoid classes have decreased their prevalence in recent samples and have resulted in synthesis of cannabinoids with increased structural diversity. In the

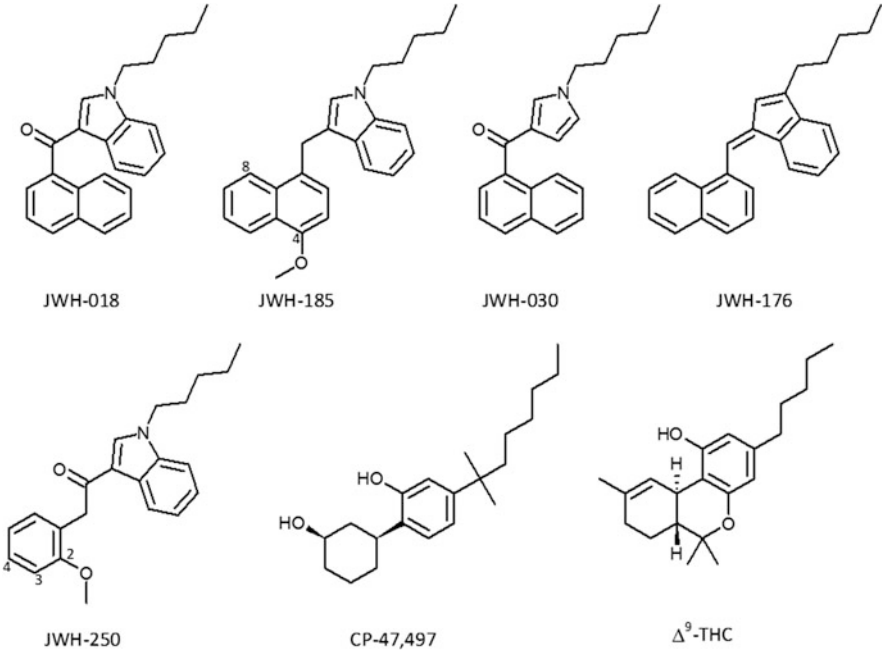

Fig. 1 Chemical structures of representative compounds from major structural groups of synthetic cannabinoids: naphthoylindoles (JWH-018), naphthylmethylindoles (JWH-185), naphthoylpyrroles (JWH-030), naphthylmethylindenes (JWH-176), phenylacetylindoles (JWH-250), cyclohexylphenols (CP-47,497), and tetrahydrocannabinols (Δ^9-THC)

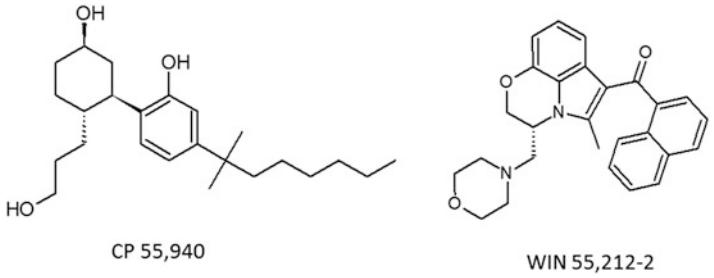

Fig. 2 Chemical structures of CP55,940 and WIN55,212-2

present chapter, a brief overview of the pharmacology of these older cannabinoids is provided, but the primary concentration is on review of the pharmacology of synthetic cannabinoids that have appeared on the market more recently.

JWH-018 (1-pentyl-3-1-naphthoylindole), a naphthoylindole, was the first cannabinoid compound identified in herbal incense products and, as such, has been referenced as the prototypic synthetic cannabinoid [33]. It is structurally similar to the aminoalkylindole WIN55,212-2 (Fig. 2), with the exception that the latter's oxazine and morpholino substituents are replaced with an *n*-pentyl group. SAR studies showed that affinity and potency varied systematically with the length of *n*-alkyl substituent, with optimal activity from *n*-butyl to *n*-hexyl and absence or reduction of receptor binding at shorter or longer carbon chains [34–36]. Replacement of JWH-018's *n*-pentyl group with *n*-fluoropentyl resulted in AM-2201, a potent psychoactive cannabinoid that appeared in confiscated samples as JWH-018 was fading in popularity [37, 38]. 2-Methylation of the indole in the alkylindole series resulted in compounds with decreased CB_1 receptor affinities and in vivo cannabimimetic potencies and a slight shift in optimal chain length. Conversion of naphthoylindoles to naphthoylpyrroles decreased CB_1 receptor affinities and reduced potencies to an even greater extent than 2-methylation [36].

While variations in the structures of early compounds focused primarily on manipulation of the alkyl group or conversion of the indole core to a pyrrole, structural innovations involving the naphthoyl group soon began to appear (e.g., JWH-185, Fig. 1). These changes included additions to and substitutions for this functional group. Additions to the naphthoyl group concentrated on alteration of the steric and electronic effects through addition of two types of substituents: electron withdrawing halogen substituents and electron donating methoxy [39, 40]. C-4 substitution of either type of substituent resulted in compounds with the best CB_1 receptor affinities and in vivo activity, as compared to substitution at other positions. Unlike substituents at other positions on the naphthoyl, the rotation of C-4 substituents is less hindered and thereby, less likely to interfere with optimal aromatic stacking, which has been shown to be important for cannabinoid receptor recognition [41, 42]. Together, these results suggest that steric effects play a

stronger role in determining the nature of CB_1 receptor affinity and in vivo activity than do electronic effects.

Steric influences and aromatic stacking are also important determinants of the effects of substitutions for the naphthoyl group. For example, SAR investigation of a series of 1-pentyl-3-phenylacetylindoles (e.g., JWH-250, Fig. 1) showed that the decrease in the number of aromatic rings on the non-indole side of the carbonyl from two (naphthoyl) to one (phenylacetyl) resulted in reduction of CB_1 receptor affinities and in vivo potencies [43]. As with addition of halogen and methoxy substituents to the naphthoyl group, the position of substituents on the phenyl ring (i.e., 2-, 3- or 4-position) also affected CB_1 receptor affinities and potencies, with 2- and 3-phenylacetyl substituents showing enhanced affinities compared to 4-substituents. Hence, steric influences are also important in binding for the 1-pentyl-3-phenylacetylindole series of synthetic cannabinoids.

Tetramethylcyclopropyl ketone indoles represent another category of synthetic cannabinoid, in which the core change is substitution of a tetramethylcyclopropyl group for the naphthoyl substituent of the parent 3-naphthoylindole. Specific compounds that have been sold over the internet include UR-144, XLR-11, and A834735 (Fig. 3) [44]. These compounds resemble those synthesized by Abbott Laboratories in their effort to develop CB_2-selective agonists for pain and inflammation [45, 46]. While many of the Abbott compounds showed higher affinity for the CB_2 receptor, a number of the compounds also possessed significant affinity for the CB_1 receptor, which undoubtedly serves as the basis for their inclusion in "herbal incense" products. All three compounds have high affinities for both CB_1 and CB_2 receptors, with XLR-11 and UR-144 having similar affinities for the CB_1 receptor ($k_i = 24$ and 29 nM, respectively) and A834735 having greater affinity ($k_i = 4.6$ nM) than the other two compounds [13, 47]. Unlike THC, both XLR-11

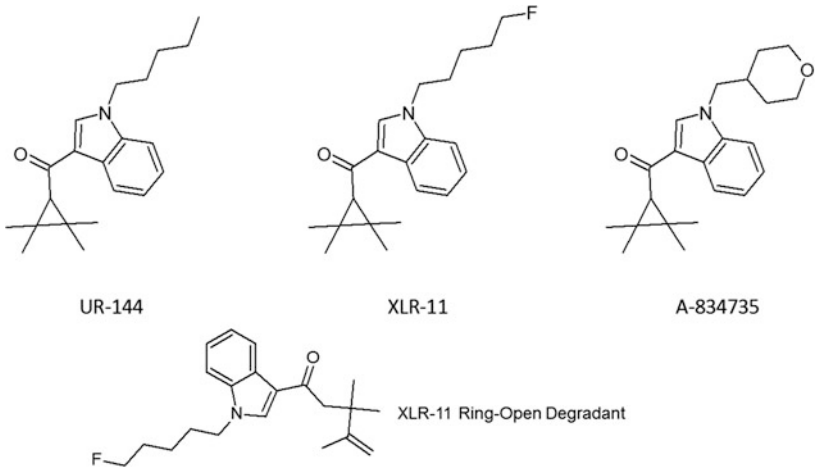

Fig. 3 Chemical structures of tetramethylcyclopropyl ketones: UR-144, XLR-11, and A-834735. Also shown is the chemical structure of the open-ring degradant of XLR-11

and UR-144 are fully efficacious CB_1 receptor agonists, as measured by GTPγS binding [13]. Further, recent data show that repeated exposure of the parent compounds to high heat (as would occur during smoking or vaping) resulted in thermolysis of the tetramethylcyclopropyl group of each compound and formation of open-ring degradants with substantially increased CB_1 receptor affinities and efficacies (Fig. 3; [48]). In human users of UR-144, analytical findings showed that the majority of urine samples contained metabolites of the pyrolysis product and only minimal amounts of the parent compound [49]. The discrepancies between chemicals contained in the product and chemicals created when the product is combusted highlight the importance of thorough analysis for accurate prediction of the effects of exposure, an idea that has been echoed in other studies [25, 44].

The tetramethylcyclopropyl substituent is only one of several novel substitutions for the naphthoyl group in products containing synthetic cannabinoids. Using a fluorometric imaging plate reader (FLIPR) assay that measures cross-membrane ion flux, Banister et al. [50–52] reported the potencies for activation of CB_1 and CB_2 receptors of several series of synthetic cannabinoids. Unfortunately, these studies did not assess binding affinities for these compounds. Since CB_1 receptor binding affinity is the single most frequent measure available across synthetic cannabinoid SAR studies [53, 54], direct integration of their results into the body of previous research is complicated. Nevertheless, several of the findings from the Australian group are of note. First, previously untested series of cannabinoids, including adamantane-derived indoles [e.g., adamantan-1-yl(1-pentyl-1H-indol-3-yl) methanone (AB-001) and N-(adamtan-1-yl)-1-pentyl-1H-indole-3-carboxamide (SDB-001); Fig. 4] and indole-3-carboxamides and -carboxylates (e.g., AB-PICA, AB-FUBICA), were shown to activate the CB_1 receptor (as measured by FLIPR) [50, 52]. Second, indole-derived compounds with a fluorine at the terminal end of the *n*-alkyl substituent were reported to exhibit more potent activation of the receptor in the FLIPR assay than those without this substitution [51]. Finally, potent in vitro activity was observed for compounds in which an indazole was substituted for the indole and in which various carboxamide and carboxylate substituents were substituted for the naphthoyl group [52]. In most cases, potencies for compounds in the indazole series exceeded those of the comparable compounds in the indole series. These results support previous work which has suggested that CB_1 receptor binding site(s) can tolerate a large degree of structural variability for agonists [55].

AB-PINACA, one of the indazoles evaluated in the Banister et al. [52] study, was assessed in additional assays in another recent paper [30]. A second indazole cannabinoid (AB-CHMINACA) and a compound with a new benzimidazole structure (FUBIMINA) were also tested (Fig. 4). Of the three compounds, FUBIMINA had the lowest CB_1 receptor affinity ($k_i = 296$ nM). While these results suggest that FUBIMINA would not be likely to show cannabinoid psychoactivity in vivo except at high doses, it has appeared in some samples confiscated in Japan [56]. Presence of the two indazole compounds in samples has also been reported [57, 58], which is not surprising given their high CB_1 receptor affinities. Of the two indazoles, AB-CHMINACA had the highest CB_1 receptor affinity ($k_i = 0.78$ nM), an affinity that was comparable to that of CP55,940 ($k_i = 0.59$ nM). Although AB-PINACA had somewhat less affinity ($k_i = 2.87$ nM), its affinity still exceeded that of the

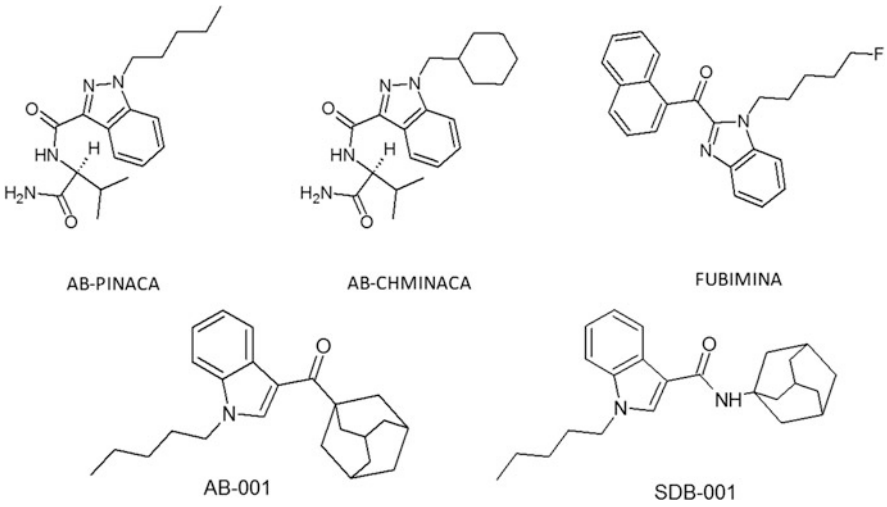

Fig. 4 Chemical structures of indazole-derived synthetic cannabinoids (AB-PINACA and AB-CHMINACA), a benzimidazole cannabinoid (FUBIMINA), and adamantane-derived cannabinoids (AB-001 and SDB-001)

prototypic phytocannabinoid THC ($k_i = 41$ nM; [59]). The sole structural difference between these two indazole cannabinoids is the substitution of a cyclohexylmethyl moiety in AB-CHMINACA for the *n*-pentyl of AB-PINACA, suggesting that receptor recognition is facilitated by the conformational restraint provided by the ring system. Results of GTPγS binding showed that both indazole and benzimidazole compounds were full agonists at CB_1 receptors [30]. Interestingly, efficacies of AB-CHMINACA and AB-PINACA for stimulation of the receptor were greater than those produced by other full agonists such as CP55,940 and WIN55,212-2 [30, 60]. In contrast, efficacies of FUBIMINA and CP55940 were comparable.

As illustrated by structural modifications present in these indazole and benzimidazole cannabinoids, structures of the most popular synthetic cannabinoids today often contain substitutions for more than one substituent. For example, compounds within the PINACA series show each of the following alterations: an indazole (vs. indole) core, lack of a cyclic structural group at the position of the naphthoyl group of the naphthoylindoles, and may contain a terminal substitution on the *n*-alkyl group [52]. This increased variety of structural modifications compared to the prototype JWH-018 is arguably the consequence of increases in the number of banned substances, highlighting the continued evolution and sophistication of synthetic cannabinoid manufacturers in response to regulation.

3.2 CB_2 Receptor

Once researchers realized that separation of CB_1 and CB_2 receptor affinity was possible, CB_2 receptor selectivity became a viable target for pharmaceutical industry investigation [61]. While a thorough review of SAR for the CB_2 receptor is beyond the scope of this chapter, several points are worth mentioning. As with most drug development efforts, determination of SAR for the target of interest often results in synthesis of many compounds that are off-target – in this case, many compounds that do not have high CB_2 selectivity. Many of these "off-target" compounds have high CB_1 receptor affinity, a property that has been exploited by manufacturers of synthetic cannabinoids contained in herbal incense products. Little information exists on the practical consequences of activation of CB_2 receptors for users of synthetic cannabinoids. For example, CB_2 receptor activation may be related to peripheral effects of synthetic cannabinoids, which have not been well characterized. On the other hand, the CB_2 receptor activating effects of synthetic cannabinoids may be enhanced in the CNS in users who have certain brain disorders or injuries (e.g., neuroinflammation) due to the proposed induction of CB_2 receptors by brain microglia under these conditions [62]. Ironically, the effects of CB_2 receptor activation, the property for which many of the currently abused compounds were originally synthesized, have received minimal research attention compared to the amount of attention that has been given to the effects of their activation of CB_1 receptors.

3.3 Noncannabinoid Receptors

Published research on synthetic cannabinoids has concentrated almost exclusively on examination of their in vitro and/or in vivo cannabinoid effects. While an occasional paper may mention lack of affinity of specific compounds for major receptor classes (e.g., [63]), for the most part, published literature on the noncannabinoid effects of synthetic cannabinoids is virtually nonexistent.

4 In Vivo Pharmacology

Although hundreds of synthetic cannabinoids have been evaluated for their CB_1 and CB_2 receptor affinities [29, 35, 39, 41, 54, 64, 65], in vivo pharmacology and toxicology studies of these compounds were rare until they were discovered in products confiscated from human users. Early studies with a limited number of compounds showed that potencies in a battery of four tests in mice (the "tetrad") were highly correlated with CB_1 receptor binding affinities [59, 66]. Psychoactive cannabinoids of various structural classes, including indole-derived cannabinoids,

produce a characteristic profile of effects in the tetrad, including suppression of locomotor activity, antinociception, hypothermia, and catalepsy [66]. They also possess THC-like discriminative stimulus effects in rodents and nonhuman primates [67, 68]. This section focuses on a review of recent in vivo studies with synthetic cannabinoids, as results of earlier in vivo studies were reviewed previously [32].

As reported in a previous review [32], XLR-11 and UR-144 produced the full complement of tetrad effects and substituted for THC in drug discrimination in mice, in each case with potencies several-fold greater than THC [13]. The tetrad effects of these two compounds were attenuated by co-administration of the prototypic CB_1 receptor antagonist rimonabant, suggesting CB_1 receptor mediation of these effects. CB_1 receptor affinities were similar for these two compounds and they possessed similar in vivo potencies. In contrast, a later investigation reported that XLR-11, a fluorine-containing analog of UR-144, showed substantially enhanced potency for activation of CB_1 receptors (compared to the non-fluorinated UR-144) and decreased body temperature at a lower dose [51]. Several differences across the studies may account for their discrepant results. First, activation of the CB_1 receptor was measured in two different assays: membrane ion flux [51] or GTPγS binding [13]. The degree to which these two preparations assess the same phenomenon is uncertain, particularly given recent findings that ligands for G-protein coupled receptors, including cannabinoid receptors [69, 70], may exhibit signaling bias [71, 72]. In contrast with functional potency in assays that measure the in vitro activation of cannabinoid receptors, receptor binding affinity (as evaluated via displacement of a radiolabeled agonist) is highly correlated with the in vivo potency of cannabinoids in the tetrad and drug discrimination procedures [36, 59, 66]. Consistent with these findings, the similar binding affinities of XLR-11 and UR-144 were predictive of their similar in vivo potencies in cannabinoid-selective procedures [13]. In vivo potencies were also calculated in a different manner across the two studies, partly as a result of different procedures. Whereas in vivo potencies in the tetrad and in THC discrimination were calculated through a least squares linear regression procedure [13], potency for producing hypothermia across time (as measured via implanted telemetric devices) was defined as the lowest dose which significantly decreased body temperature [51]. The differences between these two studies highlight the difficulty of SAR research across labs in the absence of a single common measure.

More recently, open-ring degradants of the tetramethylcyclopropyl ketones (XLR-11, UR-144, and A834735), but not a degradant of PB-22 (1-pentyl-1H-indole-3-carboxylic acid 8-quinolinyl ester), were shown to produce tetrad effects in mice and to substitute for JWH-018 in mice trained to discriminate JWH-018 from vehicle [48]. These data are consistent with anecdotal reports showing that tetramethylcyclopropyl ketone cannabinoids within this class are more potent than would be expected given affinities of the parent compounds [73].

Indazole cannabinoids, AB-CHMINACA and AB-PINACA, and the benzimidazole FUBIMINA have also been tested in vivo in the tetrad and THC discrimination procedures [30]. While AB-CHMINACA and AB-PINACA produced the full

profile of cannabinoid effects in the tetrad battery in mice, FUBIMINA was inactive except at a relatively high (56 mg/kg) intravenous dose. The effects of the three compounds also differed in THC discrimination in mice. AB-CHMINACA fully substituted for THC across a dose range that did not affect overall responding. These results are consistent with the compound's high CB_1 receptor affinity and resemble those obtained with other psychoactive cannabinoid agonists from a variety of structural classes [67, 68]. Consistent with its relatively low CB_1 receptor affinity, FUBIMINA only partially substituted for THC in mouse drug discrimination, which is also consistent with its modest CB_1 receptor affinity. The most puzzling results emerged with AB-PINACA. Although AB-PINACA produced full dose-dependent substitution for THC, this substitution was achieved only at a dose that was accompanied by substantial decreases in response rate. Previously, response rate decreases induced by other synthetic cannabinoids were observed only with doses that were suprathreshold for full substitution [12, 13]. This lack of separation between doses that are THC-like and those that substantially suppress responding suggest that AB-PINACA is a potent psychoactive CB_1 receptor agonist, but they also suggest that the doses that induce intoxication may be very close to doses associated with behavioral toxicity.

The brevity of this section on the in vivo pharmacology of synthetic cannabinoids reflects the sporadic nature of research in this area. Only a small number of the hundreds of compounds that have appeared on the illicit synthetic cannabinoid market has been tested in animals. For many of these cannabinoids, the first test subject has been human. A review of research on the toxic effects of synthetic cannabinoids follows.

5 Toxicology

Preclinical toxicological assessment of synthetic cannabinoids has been sparse, with forensic toxicology comprising the bulk of the research. Because much of the forensic research is related to identification and detection of synthetic cannabinoid metabolites, the reader is referred to the chapter on metabolism of synthetic cannabinoids for a review of relevant literature. Non-laboratory research in this area has consisted primarily of anecdotal and clinical reports and epidemiological studies, which have been reviewed previously [74, 75]. One of the primary problems with this research is the difficulty in identification of specific compounds that are associated with the various reported effects.

In general, the pharmacological effects of synthetic cannabinoids in humans resemble those of THC and may include subjective intoxication, tachycardia (fast pulse rate), and conjunctival injection ("red eyes") [76]. The degree of acute intoxication produced by synthetic cannabinoids may be more intense or milder than that produced by marijuana [77]; however, experienced marijuana users tended to prefer natural cannabis over synthetic cannabinoids [78]. After repeated use, dependence may occur [79, 80]. Differences in the clinical effects of THC and

synthetic cannabinoids have also been reported. For example, users of synthetic cannabinoids may show increased incidence (compared to marijuana) of anxiety or agitation [81], nausea and vomiting [82], hypertension [83], seizures [83, 84], and psychiatric disturbance (e.g., suicidality, exacerbation of pre-existing psychosis, and hallucinations) [79, 83, 85, 86]. Acute kidney injury may be associated with the use of XLR-11 [87, 88] and death has been known to occur as a result of synthetic cannabinoid use [89]. Consequently, synthetic cannabinoids account for a greater proportion of cannabinoid-related emergency room visits than does marijuana [90, 91].

6 Summary

In the 1980s, cannabinoid researchers developed potent synthetic cannabinoids that were used to identify the molecular and biochemical foundations of the endogenous cannabinoid system and facilitate the development of experimental therapeutics. The transition of novel synthetic cannabinoids from research chemicals to recreational use occurred in the early 2000s, increased rapidly to a multimillion-dollar designer drug industry, and continued to evolve as a public health concern despite ongoing regulatory efforts. The recreational use of synthetic cannabinoids persists in an expanding variety of chemical forms and formulations, particularly in uninformed youth, "psychonauts," and individuals attempting to avoid drug testing (e.g., military, ex-convicts, and individuals involved in public transport). Even with the current trend towards decriminalization and legalization of cannabis use, the long elimination half-life of phytocannabinoids and their metabolites constrains its recreational use in certain populations attempting to avoid detection, such that synthetic cannabinoid use continues to be of significant interest. Very little is known about the chemical purity or stability of these new chemical entities, the exposures that occur during their use, or their in vitro or in vivo pharmacological and toxicological effects. As a result, there are frequent reports of overdose and untoward effects being attributed to their use as intoxicants. The current state of affairs creates a paradoxical situation, where the potential for abuse and harm from synthetic cannabinoids must be recognized and dealt with effectively, while simultaneously enabling the development and testing of novel synthetic cannabinoids in carefully controlled preclinical and clinical studies to further elucidate the role of the endogenous cannabinoid system in health and disease states.

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