AB-PINACA

Critical Review Report

Agenda Item 4.4

Expert Committee on Drug Dependence

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Contents

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WHO would like to thank the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) for providing information on AB-PINACA from the European Union Early Warning System, which includes data reported by the Reitox National Focal Points in the EU Member States, Turkey, and Norway.

Summary

AB-PINACA (*N*-[(2*S*)-1-amino-3-methyl-1-oxobutan-2-yl]-1-pentyl-1*H*-indazole-3-carboxamide), originally developed by Pfizer Inc. as a synthetic cannabinoid receptor agonist (SCRA), has been encountered outside medical settings as a synthetic constituent and found in herbal smoking mixtures that are sold under a variety of brand names. It is common for retailers to purchase bulk quantities of the synthetic substance and add the synthetic material to plant matter that is then distributed onto the market. However, AB-PINACA is also available in powdered form as a "research chemical". The identification of AB-PINACA was reported first in 2012 by Japan and more forensic detections began to emerge in other countries in 2013. Data from law enforcement suggest that AB-PINACA was one of the most prevalent substances in the United States of America in 2014, which dropped again in the following years. A small number of *in vitro* and *in vivo* studies are currently available but the data indicate that AB-PINACA binds to and activates human CB_1 and CB_2 receptors at low nanomolar concentrations, and that it induces a number of biological responses also triggered by the naturally occurring phytocannabinoid Δ^9-THC. In tetrad assays (locomotor suppression, antinociception, hypothermia, and catalepsy), for example, AB-PINACA was shown to be 2- to 14-fold more potent than Δ^9-THC and the effects were rimonabant-reversible. In some *in vitro* assays, AB-PINACA acted as a full agonist with slight selectivity for the hCB_2 receptor. AB-PINACA also fully substituted for Δ^9-THC in the drug discrimination paradigm and was 1.5-fold more potent than the training drug, which suggests that AB-PINACA may have abuse liability similar to Δ^9-THC and/or other internationally controlled synthetic cannabinoid receptor agonists.

Reports indicate an increasing trend for SCRAs being implicated in mini epidemics that have been associated with severe adverse drug effects including deaths. Reported adverse drug reactions associated with a range of SCRAs frequently include gastrointestinal (e.g. nausea/hyperemesis), neurological (e.g. hallucination, agitation, anxiety, paranoia, confusion, delusions, catatonia, lethargy, psychosis (including susceptible individuals)), cardiovascular (e.g. tachycardia, hypertension) and renal (e.g. acute kidney failure) features. The total number of cases reported in the scientific literature that make a causal link with AB-PINACA is very small. Intoxications and deaths associated with AB-PINACA have been reported but very few details are available. 'Driving Under the Influence' cases linked to AB-PINACA intoxication and 'Drug Recognition Expert' exams revealed significant impairment. Although not specific to just AB-PINACA, there are indications that socially vulnerable and stigmatized drug users for example found in homeless and prison populations, are increasingly associated with problematic use of SCRA products. Heavy use of SCRAs has been associated with problematic withdrawal symptoms even though further research is needed to investigate the underlying mechanisms. Epidemiological data, such as prevalence of use, abuse and dependence information specifically related to AB-PINACA could not be identified.

1. Substance identification

A. International Nonproprietary Name (INN)

Not available.

B. Chemical Abstract Service (CAS) Registry Number

1445752-09-9 ((*S*)-free base)
1445583-20-9 (free base)

C. Other Chemical Names

N-[(1*S*)-1-(Aminocarbonyl)-2-methylpropyl]-1-pentyl-1*H*-indazole-3-carboxamide;
(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1*H*-indazole-3-carboxamide;
N-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-pentylindazole-3-carboxamide

D. Trade Names

Not available.

E. Street Names

AB-PINACA

F. Physical Appearance

The hydrochloride salt has been described as a white powder.[1]

G. WHO Review History

AB-PINACA has not been previously pre-reviewed or critically reviewed. A direct critical review is proposed based on information brought to WHO's attention that AB-PINACA is clandestinely manufactured, of especially serious risk to public health and society, and of no recognized therapeutic use by any party. Preliminary data collected from literature and different countries indicated that this substance may cause substantial harm and that it has no medical use.

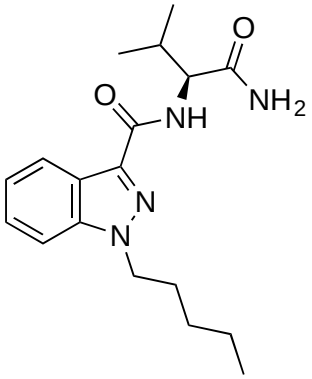
2. Chemistry

A. Chemical Name

IUPAC Name: *N*-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-pentyl-1*H*-indazole-3-carboxamide
CA Index Name: *N*-[(1*S*)-1-(Aminocarbonyl)-2-methylpropyl]-1-pentyl-1*H*-indazole-3-carboxamide

B. Chemical Structure

Free base:



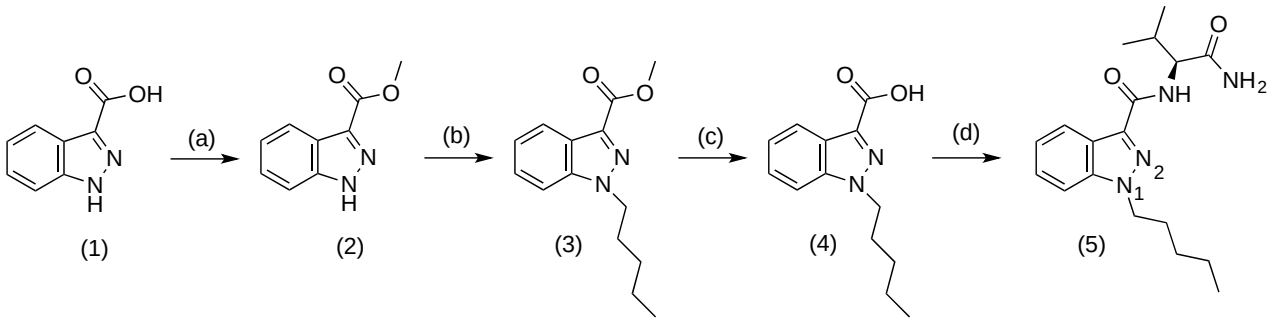
Molecular Formula: $C_{18}H_{26}N_4O_2$
Molecular Weight: 330.43 g/mol

C. Stereoisomers

The synthesis procedures described in the original patent literature[2] place an emphasis on the preparation of the (*S*)-enantiomer based on the use of L-valinamide in the last reaction step (see below). However, it is currently not known whether AB-PINACA has been exclusively found on the market with the absolute (*S*)-configuration although it appears very likely to be the case.

D. Methods and Ease of Illicit Manufacturing

The preparation of AB-PINACA is straightforward and follows standard procedures that start with indazole-3-carboxylic acid (1), which can also be prepared if not available as the starting material. Esterification (2) and N^1-alkylation (3) followed by saponification provides access to the carboxylic acid precursor (4) that is then coupled with L-valinamide to give the (*S*)-enantiomer of AB-PINACA (5).[2, 3] It was also observed that the alkylation reaction resulted in the formation of the N^2-alkylated regioisomer as an impurity, which was then shown to result in very low potency as CB_1 and CB_2 receptor agonists.[3, 4]



(a) Concentrated H_2SO_4, MeOH, reflux, 4 h; (b) 1-bromopentane, t-BuOK, THF, 0 °C to rt, 48 h, (c) NaOH, MeOH, rt, 24 h; (d) EDC HCl, HOBt, DIPEA, L-valinamide, DMF, rt, 24 h.[3, 4] The two nitrogen atoms found in the indazole ring of AB-PINACA (5) have been numbered indicate the site of alkylation. The 2*H*-regioisomer of (5) has been detected as a synthesis impurity.[3, 4]

E. Chemical Properties

Melting point: 121.8 °C (base).[1]
Boiling point: Information could not be identified.
Solubility: Information could not be identified. AB-PINACA is expected to be lipophilic in nature and therefore soluble in organic solvents.

F. Identification and Analysis

Chemical and analytical data are available. AB-PINACA has also been employed for analytical purposes and featured in a range of routine methods of analysis associated with forensic and clinical investigations (Annex 2). The analytical determination of synthetic cannabinoid receptor agonists such as AP-PINACA in biological fluids can be a challenge, for example in cases where analytical methodologies are not specifically designed to detect these substances. In some instances (e.g. analysis of urine), the detection of metabolites may be the preferred approach. Since the synthesis of AB-PINACA has been reported to produce the 2*H*-regioisomer,[3, 4] it is conceivable that it might be detectable in drug products available on the market. In this case, the analytical differentiation between these isomers is needed. The explicit identification of the (*S*)-enantiomer has not been confirmed in the reports associated with the identification of AB-PINACA. However, given that the original patent literature makes explicit reference to the evaluation of (*S*)-enantiomers[2] and that L-valinamide is cheap and widely available, it would seem likely that the AB-PINACA available on the market exists as the (*S*)-enantiomer. Until further information is available, one cannot exclude the existence of the (*R*)-enantiomer and the differentiation between the two might present challenges in routine forensic laboratories. An overview of brand names linked to the presence to AB-PINACA has been published for selected examples reported by the Customs and Border Protection Laboratory in the United States of America (period December 2013-September 2014).[5] However, it is worth noting that product compositions constantly change, which means that identical product names might not contain identical constituents at different time points.

3. Ease of Convertibility Into Controlled Substances

Information could not be identified.

4. General Pharmacology

A. Routes of administration and dosage

AB-PINACA, in its pure form but mostly as a synthetic constituent added to a plant matrix (e.g. damiana (*Turnera diffusa*) or marshmallow (*Althaea officinalis*), is normally smoked but reliable data about dosage are normally unavailable. The variations in drug composition and quantities frequently observed with many smoking mixtures (e.g.[6]) make such estimation impossible for users despite of information may be displayed on a product label.

B. Pharmacokinetics

Information from clinical studies in humans is not available. A number of reports have been published that describe the biotransformation (and/or metabolic stability) of AB-PINACA under *in vitro* conditions, either using human liver microsomes,[7-10] human hepatocytes[9, 10] or recombinant human carboxylesterases.[8] The analysis of urine samples obtained from a drug administration study in mice[11] and one involving authentic human specimen[9] has also been published. Metabolites identified following incubation with human liver microsomes included the amide hydrolysis product AB-PINACA-COOH species hydroxylated at the *N*-pentyl chain and butanamide moiety and the indazole ring. Hydroxylations were identified both at AB-PINACA and AB-PINACA-COOH.[7, 8] Furthermore, the inhibition of carboxylesterases 1 and 2 (CES) active in both human liver and pulmonary microsomes showed that the AB-PINACA hydrolysis was abolished when using the CES 1 inhibitor benzyl (at 100 μM) whereas only a 20% reduction was noticed using the selective CES 2 inhibitor loperamide.[8]

A metabolic stability study carried out with human liver microsomes suggested an *in vitro* half-life ($T_{1/2}$) of 18.7 ± 0.4 min and an *in vitro* microsomal intrinsic clearance ($CL_{int,\ micr}$) of 0.037 mL/min/mg. Scaled to whole-liver dimensions, the intrinsic clearance of AB-PINACA was estimated at 35 mL/min/kg. The calculated predicted hepatic clearance (CL_H) (without considering plasma protein binding and using a simplified Rowland's equation) was 12.7 mL/min/kg and the extraction ratio was determined as 0.6.[9]

An incubation study with human hepatocytes revealed the detection of 23 AB-PINACA metabolites (phase I and phase II) that reflected carboxamide hydrolysis, hydroxylation, ketone formation, carboxylation, epoxide formation with subsequent hydrolysis, or reaction combinations. Major metabolites included AB-PINACA-COOH, carbonyl-AB-PINACA (ketone function on *N*-pentyl chain), and hydroxypentyl AB-PINACA. Nine metabolites represented second-generation metabolites derived from AB-PINACA-COOH. A comparison with authentic human urine samples indicated that the most intense urinary metabolites (signal response under electrospray ionization conditions) reflected amide hydrolysis, additional oxidation, and often glucuronidation. Four of the five major metabolites identified in the hepatocyte assay were among the top 20 most intense metabolites in the urine specimens.[9] AB-PINACA-COOH was also detected in another urine sample collected from a user.[12] Incubation experiments with various human liver preparations (primary human hepatocytes, pooled human S9 fraction, pooled human liver microsomes combined with cytosol) uncovered extensive metabolism. A metabolite common to all samples was AB-PINACA-COOH. Two additional main species detected in an authentic human urine sample represented amide hydrolysis plus ketone formation and amide hydrolysis plus oxidation.[10]

The analysis of mouse urine samples following AB-PINACA administration revealed the detection of monohydroxylated metabolites and their glucuronides.[11] "AB-PINACA *N*-pentanoic acid" was reported as a metabolite detected in serum and urine of a 10 month old female who ingested a "K2" cigarette

unintentionally.[13, 14]

C. Pharmacodynamics

A number of *in vitro* and *in vivo* studies have been carried out which suggest that AB-PINACA, a synthetic cannabinoid receptor agonist (SCRA), binds to and activates CB_1 and CB_2 receptors, and that it induces a number of biological responses that are also triggered by the naturally occurring phytocannabinoid Δ^9-THC and other internationally controlled SCAs (Tables 1 and 2).

In vitro data – functional activity:

AB-PINACA was more potent than Δ^9-THC in the ability to activate G-protein-gated inwardly rectifying K^+ channels (GIRKs) in mouse AtT20 neuroblastoma cells transfected with human CB_1 and CB_1 receptors. AB-PINACA was 20 times (hCB_1) and 5.2 times (hCB_2) more potent than CP-55,490 and showed similar efficacy as a full agonist. In comparison to Δ^9-THC, AB-PINACA was ca. 143 times more potent. As a partial agonist, Δ^9-THC elicited 58% activation (hCB_1) compared to a 32% efficacy (hCB_2) that could only be achieved at a concentration of 30 μM. In this assay, AB-PINACA was 15 times (hCB_1) and 8.8 (hCB_2) times more potent than the full agonist JWH-018 whereas efficacy was slightly lower at hCB_1 and higher at hCB_2 (Table 1).[3] When using the [^{35}S]GTPγS turnover assay, it was reported that AB-PINACA acted as an agonist at both CB receptors. Although it was less potent than CP-55,940, an augmented efficacy was observed for AB-PINACA at hCB_1.[11] Agonist-mediated inhibition of forskolin stimulated cAMP levels monitored in HEK-293 cells expressing the hCB_1 receptor and compared relative to WIN-55,212-2, AP-PINACA was determined to be a full agonist together with the SCRAs JWH-018 and XLR-11 whereas CP-55,940 and HU-210 were identified as partial agonists. Under these assay conditions, JWH-073 was inactive (Table 1).[15] From the perspective of evaluating living cellular functional responses to drug administration, hCB_1-induced suppression of Ca^{2+} spiking was investigated in cultured rat hippocampal neurons (fluorescence detection and multi-electrode experiments). AP-PINACA induced significant overall suppression of Ca^{2+} spiking together with other SCRAs. HU-210 did not produce a suppression using the same concentration.[15] In multi-electrode experiments, AB-PINACA caused significant suppression overall at both 10 μM and 1 μM but not at all investigated time points.[16]

In vitro data – receptor binding:

Receptor binding studies in HEK-293 cells transfected with hCB_1 and hCB_2 receptors using the radioligand [^{3}H]CP-55,940 confirmed that AB-PINACA showed nanomolar affinity to both receptors with a 3.3-fold selectivity toward hCB_2. In comparison, CP-55,940 showed a 2-fold selectivity toward hCB_2 but with higher affinity than AB-PINACA at both receptors.[11] AB-PINACA also displayed a 23- and 41-fold higher affinity to hCB_1 and hCB_2 than Δ^9-THC (Table 1).[17]

Table 1. AB-PINACA in-vitro data	**Ref**
Functional activity: [a] AB-PINACA at hCB_1: EC_{50} = 1.2 nM (E_{max} = 103%); hCB_2: EC_{50} = 2.5 nM (E_{max} = 104%) Δ^9-THC at hCB_1: EC_{50} = 172 nM (E_{max} = 58%); hCB_2: EC_{50} not determined; too low (E_{max} = 32% at 30,000 nM) CP-55,490 at hCB_1: EC_{50} = 24 nM (E_{max} = 100%); hCB_2: EC_{50} = 13 nM (E_{max} = 100%) JWH-018 at hCB_1: EC_{50} = 18 nM (E_{max} = 116%); hCB_2: EC_{50} = 22 nM (E_{max} = 87%)	Banister et al.[3]
Receptor binding: Details not reported CB_1: K_i = 2.64 nM ; 0.93 nM Functional activity: No details reported EC_{50} = 14.3 nM	DEA[5]
Receptor binding: [b] AB-PINACA at hCB_1: K_i = 2.87 nM ([^{3}H]CP-55,940); hCB_2: K_i = 0.88 nM ([^{3}H]CP-55,940). CP-55,940 at hCB_1: K_i = 0.59 nM ([^{3}H]CP-55,940); hCB_2: K_i = 0.30 nM ([^{3}H]CP-55,940). Δ^9-THC (data from previous study) at hCB_1: K_i = 67 nM ([^{3}H]CP-55,940); hCB_2: K_i = 36 nM ([^{3}H]CP-55,940).[17] XLR-11 (data from previous study) at hCB_1: K_i = 24 nM ([^{3}H]CP-55,940); hCB_2: K_i = 2.1 nM ([^{3}H]CP-55,940).[17] UR-144 (data from previous study) at hCB_1: K_i = 29 nM ([^{3}H]CP-55,940); hCB_2: K_i = 4.5 nM ([^{3}H]CP-55,940).[17] [^{35}S]GTPγS turnover: [c] AB-PINACA at hCB_1: EC_{50} = 71 nM (E_{max} = 192%); hCB_2: EC_{50} = 14.9 nM (E_{max} = 41%). CP-55,940 at hCB_1: EC_{50} = 23.3 nM (E_{max} = 124%); hCB_2: EC_{50} = 2.1 nM (23 nM in ref[17]) (E_{max} = 63%). XLR-11 (data from previous study) at hCB_1: ED_{50} = 159 nM ("full agonist"); hCB_2: ED_{50} = 145 nM ("full agonist").[17] UR-144 (data from previous study) at hCB_1: ED_{50} = 98 nM ("full agonist"); hCB_2: ED_{50} = 334 nM ("full agonist").[17]	Wiley et al.[11]
Functional activity (hCB_1): [d] Inhibition of forskolin stimulated cAMP levels: Efficacy relative to WIN-55,212-2: EC_{50} = 79 nM (E_{max} = 65%). AB-PINACA: EC_{50} = 79 nM (E_{max} = 69%), full agonist. CP-55,940: EC_{50} = 316 nM (E_{max} = 47%), partial agonist. HU-210: EC_{50} = 1585 nM (E_{max} = 35%), partial agonist. JWH-018: EC_{50} = 251 nM (E_{max} = 55%), full agonist. JWH-073: no activity. XLR-11: EC_{50} = 3981 nM (E_{max} = 65%), full agonist. 5F-PB-22: EC_{50} = 39.8 nM (E_{max} = 67%), full agonist. hCB_1-induced suppression of Ca^{2+} spiking in cultured rat hippocampal neurons: Compared to addition of blank. AB-PINACA: significant suppression of Ca^{2+} spiking at 10 μM. WIN-55,212-2: significant suppression at 10 μM. CP-55,940: significant suppression at 10 μM. HU-210: no suppression at 10 μM. XLR-11: significant suppression at 1 μM and10 μM (significant more suppression than AB-PINACA) 5F-PB-22: significant suppression at 10 μM.	Costain et al.[15]

Functional activity (hCB_1): [e] hCB_1-induced suppression of Ca^{2+} spiking in cultured rat hippocampal neurons (multi-electrode experiments): Percent change in number of spontaneous spikes before and after addition of 0.1% DMSO. AB-PINACA caused suppression overall at both 10 μM and 1 μM but not at all investigated time points. WIN-55,212-2 (10 μM), WIN-55,212-3 (10 μM), HU-210 (10 μM), CP-55,940 (10 μM) suppressed overall activity. JWH-018 (10 μM and 1 μM), XLR-11 (10 μM), JWH-250 (10 μM), 5F-PB-22 (10 μM), and MAM-2201 (10 μM and 1 μM) suppressed overall activity. "Overall" suppression determined at the end of the recording time. For some compounds, suppression was not always considered significant at a number of earlier time points, thus, indicating suppression occurring at longer incubation times. Rimonabant (5 μM) significantly reversed 5F-PB-22-induced suppression (10 μM) (AB-PINACA was not tested) at the first three time-matched recordings and overall.	Tauskela et al.[16]

[a] Ref[3]: hCB_1 and hCB_2 receptors in stably transfected mouse AtT20 neuroblastoma cells; FLIPR membrane potential assay (blue) used for quantitative determination of K^+ flux (hyperpolarization) linked to G-protein activation: G-protein-gated inwardly rectifying K^+ channels (GIRKs); plates were incubated at ambient CO_2 for 45 min at 37 °C; receptor activation and stimulation of cellular hyperpolarization led to decrease in fluorescence response. Comparison of test drugs was normalized against CP-55,940 response (set at 100% efficacy).

[b] Ref[11]: hCB_1 and hCB_2 (HEK-293); [^{3}H]CP-55,940 (0.62 nM) used for displacement (nonspecific binding determined by inclusion of 10 μM unlabeled CP-55,940).

[c] Ref[11]: incubation of mixture containing test drug (0.25 nM to 20 μM), GDP (20 μM), GTPγ[^{35}S] (100 pM), and hCB_1 and hCB_2 membrane preparations from HEK-293 cells. Basal binding determined in the absence of test drug; E_{max} values represent percentage of maximal increase for [^{35}S]GTPγS binding over basal.

[d] Ref[15]: GloSensor™ cAMP assay; agonist-mediated inhibition of forskolin-stimulated cyclic adenosine monophosphate (cAMP) levels was monitored in live HEK293T cells transfected with human cannabinoid receptor 1 gene (CNR1) and pGloSensor-22F; efficacy determinations (%inhibition) relative to full agonist WIN-55,212-2. Synthetic cannabinoids were added 12 min prior to the addition of 10 μM forskolin. Luminescence was determined 15 min after forskolin addition; data were normalized to vehicle readings. Low-density primary hippocampal neuron cultures were loaded with a Ca^{2+} indicator and exposed to low Mg^{2+} buffer to induce spontaneous, transient increases in intracellular Ca^{2+} levels (Ca^{2+} spikes). Post-drug frequencies were normalized to pre-drug frequencies and post-drug % inhibition was calculated relative to the pre-drug condition.

[e] Ref[16]: Hippocampal neurons plated at high density; each multi-electrode array served as its own internal control: two 20 min baseline recordings were performed prior to acquiring four 20 min recordings with a cannabinoid or DMSO vehicle present; online extracellular spike detection was used; activity was monitored over a total 80 min exposure of a cannabinoid, in 20 min recording sessions.

In vivo data:

Currently available data suggest that AB-PINACA induces responses in animals that are also seen with Δ^9-THC and other SCRAs, such as suppression of locomotor activity, decreased body temperature, antinociception, and catalepsy (tetrad assays). For example, in the tetrad assays, AB-PINACA was 2- to 14-fold more potent than Δ^9-THC. In addition, these effects were rimonabant reversible.[11] Furthermore, AB-PINACA was found to fully substitute for Δ^9-THC using the drug discrimination paradigm in mice (nose poke response) although this was only achieved at a 3 mg/kg and accompanied by substantial decreases in response rate, with only a small percentage of mice responding (Table 2). Another documented difference to Δ^9-

THC was the observation of short-lived convulsive behavior.[11] In one study, rimonabant was observed to partially reverse hypothermic effects in male Wistar rats whereas administration of a CB_2 receptor antagonist was considered ineffective.[3] A number of acute and long-lasting (residual) behavioral and neurochemical effects were investigated in adolescent rats following AB-PINACA and Δ^9-THC administration (low dose and high dosing regimen), which resulted in somewhat comparable observations (Table 2). Acute effects observed were reduction in locomotor activity, inhibition of weight gain, and increased anxiety-like behaviors. The assessment of residual effects revealed that Δ^9-THC but not AB-PINACA was associated with impairment of social interaction, whereas recognition memory impairment was observed for both drugs (2 weeks).[18] Microdialysis studies (nucleus accumbens) in male Sprague–Dawley rats revealed weak fluctuating changes in dopamine and serotonin levels where statistical significance was only reached at individual time points, mostly below the 200% level (Table 2).[19]

Table 2. *In vivo* assay data for AB-PINACA

Behavior / physiology / neurochemistry	**Ref**
Body temperature: [a] AB-PINACA: hypothermic effect (0.3–3 mg/kg) (> 1.5 °C) was observed. Maximum drop in body temperature observed with in the first hour of the 6 h monitoring period. Rimonabant partially reversed hypothermic effects but CB_2 receptor antagonist SR-144528 had only negligible effects on drug-induced hypothermia. Heart rate: [a] AB-PINACA: significant decrease in heart rate but more variable than body temperature data, possibly reflecting multiple determinants. Rimonabant reduced AB-PINACA-induced bradycardia but without reaching statistical significance. SR-144528 pre-treatment did not have any effects.	Banister et al.[3]
Tetrad tests: [b] Inhibition of spontaneous activity (locomotor activity): AB-PNACA: ED_{50} = 7.6 μmol/kg Δ^9-THC: ED_{50} ED_{50} = 104 μmol/kg Drop in total counts compared to vehicle condition with significant difference: AB-PINACA: 10 and 30 mg/kg Δ^9-THC: not reached with dose up to 56 mg/kg Percent maximum possible antinociceptive effect (tail-flick): AB-PINACA: ED_{50} = 13.7 μmol/kg Δ^9-THC: ED_{50} = 34 μmol/kg Compared to vehicle condition with significant difference: AB-PINACA: 10 and 30 mg/kg Δ^9-THC: 56 mg/kg Rectal temperature (hypothermia): AB-PINACA: ED_{50} = 5.3 μmol/kg Δ^9-THC: ED_{50} = 30 μmol/kg	Wiley et al.[11]

Drop in rectal temperature compared to vehicle condition with significant difference: AB-PINACA: 3, 10 and 30 mg/kg Δ^9-THC: 10 and 56 mg/kg Ring immobility (catalepsy): AB-PINACA: ED_{50} = 13.9 μmol/kg Δ^9-THC: ED_{50} = 30 μmol/kg Percentage increase in immobility compared to vehicle condition with significant difference: AB-PINACA: 3, 10 and 30 mg/kg Δ^9-THC: 3, 10 and 56 mg/kg Intraperitoneal doses of 56 mg/kg Δ^9-THC and 30 mg/kg AB-PINACA (injected 10 min prior to test drug) significantly suppressed locomotor activity and produced antinociception, hypothermia and catalepsy, which were attenuated by co-administration of 3 mg/kg (i.p.) rimonabant. In addition to effects on tetrad assays, 30 mg/kg AB-PINACA induced convulsions, flattened body posture (splayed limbs), and labored breathing in most mice within 1 minute after i.p. injection. Mice started to recover by the end of tetrad testing. Drug discrimination (lever and nose poke response): [c] AB-PINACA: ED_{50} not reported (lever); ED_{50} = 3.78 μmol/kg (nose poke) Δ^9-THC: ED_{50} = 4.5 μmol/kg (lever); ED_{50} = 5.7 μmol/kg (nose poke) AB-CHMINACA: ED_{50} not reported (lever); ED_{50} = 0.34 μmol/kg (nose poke) AB-PINACA substituted fully and dose-dependently for Δ^9-THC, but full substitution was achieved only at a dose (3 mg/kg) that was accompanied by substantial decreases in response rate, with only a small percentage (22%) of mice responding at this dose. It was suggested that the doses used may have been too close to differentiate between intoxication and behavioral toxicity, as reflected by a steep response rate dose-effect function (no effect at 1.7 mg/kg vs nearly complete suppression at 3 mg/kg). In contrast, response rates were significantly increased by 3 mg/kg Δ^9-THC.	
Acute and long-lasting drug effects on rats: [d] Acute effects: Significant overall effect of ABPINACA on locomotor activity (low and high dose). Δ^9-THC induced significant reduction only at high dose. In adolescent rats, AB-PINACA and Δ^9-THC produced locomotor suppression, anxiogenic effects (high dose) in the emergence test, increased audible vocalizations during handling (high dose) (supplementary measure of drug aversion) and inhibition of body weight gain (adult rats not affected); conditioned place aversion was not observed. Residual effects following 2 week washout period: [d] Novel object recognition test (% reduction): Significant overall effect of APINACA and Δ^9-THC with inter-trial interval of 60 min but not at 2 min interval. Behavior/social interaction 19 days post-administration: Exploratory and social behavior scores did not reach statistical significance with AB-PINACA but total interaction scores were significantly reduced with Δ^9-THC. Post-mortem plasma and cerebellum analyses: In plasma, levels of steroids, cytokines and ethanolamides remained unaffected by AB-PINACA and Δ^9-THC. In the cerebellum, AB-PINACA induced significant reductions in anandamide, palmitoylethanolamide, and oleoylethanolamide concentrations. This was also observed with Δ^9-THC, which was also correlated with reduced 2-arachidonoylglycerol concentrations in the same tissue.	Kevin et al.[18]

Microdialysis (nucleus accumbens): [e] AB-PINACA. 5-HT increase: <200% (80 and 120 min, $p<0.1$ and 180 min, $p<0.05$). DA increase: >200% (180 min, $p<0.01$).	Kim et al.[19]
Conditioned place preference: [f] More time spent in drug-paired chamber (0.1 mg/kg AB-PINACA) Self-administration: [f] Results not reported / self-administration not observed? Behavioral sensitization: [f] Results not reported / sensitization not observed? Motor coordination: [f] Reduced rotarod performance compared to vehicle at 30 and 40 mg/kg. Behavioral tests: [f] AB-PINACA: Significant effects on Y-maze test at 10 mg/kg but not at 20 mg/kg. AB-PINACA: Reduced exploration of novel objects at 30 and 40 mg/kg. AB-PINACA: Reduced latency in passive avoidance test 30 and 40 mg/kg.	Cha[20]

[a] Ref[3]: Male Wistar rats; biotelemetry transmitters placed in the peritoneal cavity; drugs administered (i.p.) in an ascending dose sequence (0.1, 0.3, 1, 3 mg/kg) (10 mg/kg if required) at the same time of day; data for heart rate and body temperature gathered at 1000 Hz (15 or 30 min bins). Data were recorded for 6 h post-injection. For antagonist studies (30 min before test drug treatment), each cohort received injections of vehicle, CB_1 antagonist (rimonabant, 3 mg/kg), or CB_2 antagonist (SR-144528, 3 mg/kg), followed by 3 mg/kg AB-PINACA.

[b] Ref[11]: Male ICR mice; intraperitoneal injections of vehicle, Δ^9-THC and AB-PINACA 30 min before test sessions; spontaneous activity measured for 10 min; tail-flick (radiant heat, intense light) and rectal temperature tested 30 min post-injection; ring immobility tested at 50 min post-injection.

[c] Ref[11]: Male C57/BL6J mice; trained to respond on one of the two levers following intraperitoneal (i.p.) administration of 5.6 mg/kg Δ^9-THC and to respond on the other lever following i.p. vehicle injection according to a fixed ratio 10 (FR10) schedule of food reinforcement, under which 10 consecutive responses on the correct (injection-appropriate) lever resulted in delivery of a food pellet; 15 min daily training sessions were held until: a) the first completed FR10 was on correct lever; b) >80% of total responding on correct lever, and c) response rate >0.17 responses/s. Additional mice were trained de novo on the nose poke response.

[d] Ref[18]: Male albino Wistar rats; test drugs injected (i.p.) using a low and high dose regimen: AB-PINACA every second day (commencing post-natal day 31), first at 0.2 mg/kg on 6 days followed by 1 mg/kg on a further 6 days. Δ^9-THC-treated rats received equivalent doses of 6 × 1 mg/kg and 6 × 5 mg/kg. Behavioral assessment conducted over two phases: 'on-drug' phase (behavior measure 15 min post-injection) during repeated drug administration (adolescence, PNDs 31–55), and a 'residual' phase following a 2-week washout during adulthood (PNDs 69–94).

[e] Ref[19]: Male Sprague–Dawley rats; microdialysis probe inserted into nucleus accumbens shell; six baseline samples collected following microdialysates every 20 min for 2 h. Test drug injected (i.p.) with increasing doses (0.3 at time zero, 1 at 60 min and 3 mg/kg at the 120 min point) and microdialysates were collected at 20 min intervals; analysis of DA, 5-HT, DOPAC, HVA and 5-HIAA done by LC-MS/MS.

[f] Ref[20]: Male C57/BL6 mice used for conditioned place preference (0.01, 0.03, 0.1 mg/kg, i.p., unbiased method; animals not selected after pre-tracking), sensitization (change in locomotor activity across injections), motor function and memory tests; male Sprague-Dawley rats used for self-administration and microdialysis studies; Y-Maze tests, novel object recognition tests and passive avoidance tests were used to assess exploratory behavior/learning/memory.

5. Toxicology

During the evaluation of mouse tetrad effects (Table 2), AB-PINACA (30 mg/kg, i.p.) was observed to induce convulsions, flattened body posture (splayed limbs), and labored breathing in most mice within 1 minute. The mice recovered by the end of the tetrad

assays. During drug discrimination studies, a steep response rate dose-effect was observed.[11] Chronic administration of AB-PINACA (Table 2) produced statistically significant inhibition of weight gain in adolescent rats. Total social interaction scores were not affected compared to vehicle although this was the case with Δ^{9}-THC-treated rats.[18] Any other information related to acute and chronic preclinical toxicology could not be identified.

6. Adverse Reactions in Humans

Reports associated with the presence of AB-PINACA in drug products and biofluids is summarized in Table 3 below. The total number of cases reported in the scientific literature that make a specific causal link with AB-PINACA is small.

Reported adverse drug reactions associated with a range of synthetic cannabinoids frequently include gastrointestinal (e.g. nausea/hyperemesis), neurological (e.g. hallucination, agitation, anxiety, paranoia, confusion, delusions, catatonia, lethargy, psychosis (including susceptible individuals)), cardiovascular (e.g. tachycardia, hypertension) and renal (e.g. acute kidney failure) features.[21-23]

Table 3. Case reports associated with the involvement of AB-PINACA reported in the scientific literature.

Year	Cases	Patient, age	Comments (examples)	Ref
2013	1	M, 40	Death following a head on motor vehicle collision. Toxicology results of the opposing driver detected AB-PINACA and AB-FUBINACA in biological samples.	DEA[5, a]
2014	2	M, 19 M, 37	Two deaths involving AB-PINACA; cause of death in both cases deemed synthetic cannabinoid-related.	DEA[5]
2014	1	M, NR [b]	Male presented with excited delirium; AB-PINACA detected on drug evidence.	DEA[5]
2014	2	M, 16 F, 17	Both experienced delirium after ingesting liquid drug form through hookah pen; AB-PINACA detected on drug evidence.	DEA[5]
2014	3	F, 15 F, 15 F, 17	Patients suffered loss of consciousness and seizures following ingestion of a synthetic cannabinoid product. AB-PINACA and AB-CHMINACA detected on drug evidence.	DEA[5]
2014	4	NR, NR	Four juveniles taken to emergency department following ingestion of e-liquid identified as "Cloud 9"; AB-PINACA detected in product.	DEA[5]
"Clusters of cases of adverse health effects or severe toxic effects and deaths associated with synthetic cannabinoid product use." Analysis of clinical samples; details not reported. Locations: USA August 2014: 3 cases (AB-PINACA and AB-CHMINACA detected) (St. Louis, MO). August 2014: >44 cases (AM-2201 *N*-(3-chloropentyl) isomer, AB-PINACA, ADB-PINACA, UR-144 detected) (Manchester, NH). September 2014: 6 cases (AB-PINACA detected) (Westland, MI). December 2014-January 2015: >62 cases (MAB-CHMINACA, AB-PINACA, ADB-PINACA detected) (Beaumont, TX, USA). [c] June 2014: 2 cases (AB-FUBINACA, AB-PINACA detected) (Atlantic City, NJ). August 2014: 1 case and 1 death (THJ-2201, AB-PINACA detected) (Austin, TX). October 2014: 1 case (AB-PINACA detected) (Holdrege, NE). October 2014: 1 case and 1 death (AB-CHMINACA, AB-PINACA, ADB-PINACAMAB-CHMINACA detected) (Austin, TX).				Trecki et al.[24]
2015	25	Male 95% Mean age 28; Range	Review of 58 suspected impaired driving cases submitted to a laboratory in 2014. Twenty-five cases involved AP-PINACA with blood concentrations ranging between 0.6-41.3 ng/mL (other synthetic cannabinoids detected in 5 cases). Ten cases: Drug Recognition Expert exams: horizontal gaze nystagmus observed (n = 5 at low blood concentrations); lack of convergence (n = 7); low	Peterson and Couper[25]

		18-61	blood pressure (<120/70 mm Hg) (n = 5); watery eyes and droopy eyelids observed n = unclear). Fifteen cases: Driving Under the Influence; erratic driving (n = 7); individual slumped over wheel or unconscious in the vehicle upon initial contact (n = 4); drivers stopped in a roadway (n = 5); cases of collisions (n = 6).	
2015	1	F, 10 months	Ten months old female ingested less than half a cigarette; serum levels: 42 ng/mL AB-PINACA and 345 ng/mL AB-PINACA *N*-pentanoic acid; within 90 min of ED presentation, child stopped responding to verbal and physical stimuli and developed respiratory depression requiring intubation; patient was admitted and tested positive for influenza A and treated with oseltamivir, but aspiration pneumonia complicated her course; patient was intubated for 36 h and recovered. Serum values of the acid metabolite was detected at 232 ng/mL (serum) 12 h after presentation and 1.7 µg/mL levels in urine 16 h after presentation.	Thornton et al.[13]
2017	1	M, NR	Motor vehicle collision case; details not reported (presumably from 2014); blood concentration of AB-PINACA = 0.04 ng/mL.	Kaneko[26]
[a] Details not reported; statements taken verbatim from reference. It is unclear whether there is an overlap with the case summary provided by Trecki et al.[24] [b] NR: not reported [c] "Substance identified on the basis of information about local law-enforcement seizures during the same period."				

7. Dependence Potential

A. Animal Studies

No information available.

B. Human Studies

No information available.

8. Abuse Potential

A. Animal Studies

As summarized in Tables 1 and 2 (Section 4C), AB-PINACA was shown to share some cannabinoid-like properties (e.g. binding to and activation of CB_1 and CB_2 receptors). AB-PINACA was reported to induce (rimonabant-reversible) cannabinoid-like responses in the tetrad test battery. One drug discrimination study uncovered that AB-PINACA (more potently) substituted fully for Δ^9-THC but the difference to Δ^9-THC and some other synthetic cannabinoid receptor antagonists (e.g.[27]) was that full substitution was only achieved in two out of nine mice,[11] which led to the suggestion that the dose needed to impair behavior/induce intoxication and the dose needed to produce behavioral toxicity might be very close or even indistinguishable (Table 2).[11] These data suggest that AB-PINACA may have abuse liability and further studies are warranted to investigate this further.

B. Human Studies

No information available.

9. Therapeutic Applications and Extent of Therapeutic Use and Epidemiology of Medical Use

AB-PINACA is not known to have any therapeutic applications.

10. Listing on the WHO Model List of Essential Medicines

AB-PINACA is not listed on the WHO Model List of Essential Medicines (20th List) or the WHO Model List of Essential Medicines for Children (6th List).

11. Marketing Authorizations (as a Medicinal Product)

AB-PINACA is not marketed as a medicine.

12. Industrial Use

AB-PINACA has no reported industrial use.

13. Non-Medical Use, Abuse and Dependence

Household or subpopulation surveys that specifically probe for prevalence of AB-PINACA could not be identified in the currently available literature. Epidemiological data, such as prevalence of use, abuse and dependence information, are not available specifically for AB-PINACA. However, heavy use of synthetic cannabinoid receptor agonists has been associated with problematic withdrawal symptoms (e.g.[28, 29]) and further research is needed to investigate the underlying mechanisms.

14. Nature and Magnitude of Public Health Problems Related to Misuse, Abuse and Dependence

The majority of available synthetic cannabinoid products (including those containing AB-PINACA) is sold in the form of herbal mixtures, and designed for smoking purposes. It is common for retailers to purchase bulk quantities of the synthetic substance and to add the synthetic material to a variety of vegetable matter as the plant base. Products sold as herbal smoking mixtures frequently change in drug composition and quantity, often without indications on product labels, which results in challenges to unambiguously correlate harms to public health with a specific drug such as AB-PINACA.

The consumption of these products might be attractive to a variety of users, such as regular users of cannabis and those who might wish to avoid drug-testing procedures resulting in positive cannabis findings. Ease of access, and perceived lack of control might equally be of interest to some users. The high potency associated with many synthetic cannabinoids carries the risk of accidental overdose and potentially severe adverse events but

information specific to AB-PINACA is limited. As highlighted in Section 6 (Table 3), the ingestion of AB-PINACA products has been implicated in cases of impaired driving and motor vehicle collisions,[25, 26] which might add a public health dimension. Although not specific to AB-PINACA, there are indications that socially vulnerable and stigmatized drug users, for example found in homeless and prison populations, are increasingly associated with problematic use of synthetic cannabinoid receptor agonist products.[30-33]

15. Licit Production, Consumption and International Trade

AB-PINACA is available as standard reference material and produced for scientific research by a number of commercial suppliers. Other uses could not be identified.

16. Illicit Manufacture and Traffic and Related Information

Reports have been received from the European Early-Warning System on new psychoactive substances that AB-PINACA (first detected in 2013) was encountered in seizures and collected specimen (herbal mixtures or powders) in Sweden, Hungary, Turkey, Germany, France, Croatia, Latvia, Bulgaria, Slovakia, Spain and Slovenia.[34]

AB-PINACA detections started to appear in United States of America (USA) in 2013. The National Forensic Laboratory Information System (NFLIS), which is dedicated to the collection of drug cases submitted by State and local laboratories in the USA, registered a particular increase in 2014 (Table 4), which has also been exemplified in the fact that ABPINACA did not feature in the NFLIS special report covering the 2010-2013 time period.[35] In 2014, AB-PINACA and AB-FUBINACA were the most commonly reported synthetic cannabinoid receptor agonists but this figure dropped since 2015, which reflected the emergence of new replacements,[36, 37] presumably as a consequence of issuing an order to temporarily schedule AB-PINACA into Schedule I of the Controlled Substances Act.[38, 39]

Table 4. Number of reports received by the U.S. National Forensic Laboratory Information System (NFLIS) related to detections of AB-PINACA in law enforcement operations.

Year [a]	Numbers	XLR-11	MDMA	Meth [b]	Ref
2013 (MY)	--	11,273	2,423	100,045	NFLIS[40]
2013 (AR)	965	19,243	4,798	206,784	NFLIS[41]
2014 (MY) [c]	2,168	6,316	2,224	117,318	NFLIS[42]
2014 (AR)	4,954	11,001	4,902	236,175	NFLIS[43]
2015 (MY)	1,893	3,769	2,421	133,374	NFLIS[44]
2015 (AR)	2,493	6,973	5,188	277,823	NFLIS[45]
2016 (MY)	243	1,409	2,901	155,535	NFLIS[37]

[a] MY: mid-year report (January to June); AR: annual report (January to December).
[b] Meth: methamphetamine.
[c] Revised March 2016.

Detections of AB-PINACA have also been reported to UNODC's Early Warning Advisory on New Psychoactive Substances.[46] AB-PINACA was reported by one country (Japan[47]) in 2012, 19 countries in 2013, 21 countries in 2014, 18 countries in 2015 and 7 countries in 2016 (as of 08 August 2017).

17. Current International Controls and Their Impact

AB-PINACA is not controlled under the 1961, 1971 or 1988 United Nations Conventions.

18. Current and Past National Controls

Refer to Annex 1: Report on WHO questionnaire for review of psychoactive substances.

19. Other Medical and Scientific Matters Relevant for a Recommendation on the Scheduling of the Substance

Not applicable.

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Annex 1: Report on WHO Questionnaire for Review of Psychoactive Substances for the 39th ECDD: Evaluation of AB-PINACA

Please refer to separate Annex 1 document published on ECDD website

Annex 2: Studies associated with the detection and chemical analysis of AB-PINACA (amongst other substances) published in the scientific literature.

Techniques [a]	Comment	Reference
Melting point, ^{1}H-NMR, GC-MS	Characterization of reference material.	SWGDRUG[1]
LC-UV/DAD, LC-MS, NMR, DART-TOF-MS	Identification in samples purchased on the Internet in July 2012 in Japan.	Uchiyama et al.[2]
Not reported	Review of seized products in South Korea.	Chung et al.[3, 4]
^{1}H-NMR	Analysis of reference material.	Huang et al.[5]
LC-QqQ-MS/MS	*In vitro* metabolism studies using human liver microsomes.	Takayama et al.[6]
NMR, ESI-MS	Synthesis and use in pharmacological investigations.	Banister et al.[7]
LC-QTOF-MS/MS	Analysis of urine samples.	Labutin et al.[8]
LC-QqQ-MS/MS	Analysis of blood samples from suspected impaired driving cases.	Peterson et al.[9]
LC-QTOF-MS/MS	Method development and analysis of authentic urine samples.	Scheidweiler et al.[10]
LC-Q-Orbitrap-MS/MS	*In vitro* metabolism studies using human liver microsomes and recombinant carboxylesterases.	Thomsen et al.[11]
LC-QTOF-MS	Serum analysis of accidental exposure in 10-moth old girl.	Thornton et al.[12, 13]
Not reported	Summary of cases related to acute intoxications.	Trecki et al.[14]
LC-QTOF-MS/MS	Analysis of urine samples obtained from mice.	Wiley et al.[15]
LC-QqQ-TOF-MS	*In vitro* metabolism studies using human liver microsomes, human hepatocytes and analysis of authentic urine samples.	Wohlfarth et al.[16]
GC-MS, LC-TOF-MS	Characterization of reference material.	Akamatsu et al.[17]
Cyclic and differential pulse voltammetry, GC-MS, LC-QTOF-MS	Electrochemical characterization of reference material.	Dronova et al.[18]
Immunodetection	Antibody binding characteristics of reference material	Fitzgerald et al.[19]
Melting point, TLC, NMR, ESI-HR-MS; IR, UV, GC-MS	Synthesis and characterization for pharmacological studies.	Longworth et al.[20]
LC-QTOF-MS/MS	Analysis of collected samples.	Bijlsma et al.[21]
LC-QqQ-MS/MS	Stability study in whole blood.	Fort et al.[22]
Not reported	Review of impaired driving cases.	Kaneko[23]
LC-QTRAP-MS/MS	Detection in urine samples obtained from fatalities.	Minakata et al.[24]
LC-Q-Orbitrap-MS/MS	*In vitro* metabolism studies using various human liver preparations and comparison with authentic urine specimen.	Richter et al.[25]
LC-QqQ-MS/MS	Method development and application to analysis of blood samples.	Tynon et al.[26]

[a] NMR: nuclear magnetic resonance spectroscopy; GC: gas chromatography; MS: mass spectrometry; ATR-FTIR: attenuated total reflection Fourier transform infrared spectroscopy; LC: liquid chromatography (various forms); UV: ultraviolet; DAD: diode array detector; DART: direct analysis in real time; TOF: time-of-flight; QqQ: triple quadrupole; MS/MS: tandem mass spectrometry; ESI: electrospray ionization; Q: quadrupole; QTRAP: quadrupole-linear ion trap; TLC: thin-layer chromatography; HR: high resolution.

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(AB-PINACA) and N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide (AB-FUBINACA) identified as designer drugs in illegal products. *Forensic Toxicol* 2013;31:93-100. doi:10.1007/s11419-012-0171-4.

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