Review Article

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Overview of the major classes of new psychoactive substances, psychoactive effects, analytical determination and conformational analysis of selected illegal drugs

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Abstract: The misuse of psychoactive substances is attracting a great deal of attention from the general public. An increase use of psychoactive substances is observed among young people who do not have enough awareness of the harmful effects of these substances. Easy access to illicit drugs at low cost and lack of effective means of routine screening for new psychoactive substances (NPS) have contributed to the rapid increase in their use. New research and evidence suggest that drug use can cause a variety of adverse psychological and physiological effects on human health (anxiety, panic, paranoia, psychosis, and seizures). We describe different classes of these NPS drugs with emphasis on the methods used to identify them and the identification of their metabolites in biological specimens. This is the first review that thoroughly gives the literature on both natural and synthetic illegal drugs with old known data and very hot new topics and investigations, which enables the researcher to use it as a starting point in the literature exploration and planning of the own research. For the first time, the conformational analysis was done for selected illegal drugs, giving rise to the search of the biologically active conformations both theoretically and using lab experiments.

Keywords: illegal drugs, cathinones, phenethylamines, cannabinoids, tryptamines

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Abbreviations

NMR	nuclear magnetic resonance
MS	mass spectrometry
NPS	new psychoactive substances
USA	United States of America
GABA	γ-aminobutyric acid
EU	European Union
LSD	lysergic acid diethylamide
LSA	lysergamide
UK	United Kingdom
DMT	*N*,*N*-dimethyltryptamine
NE	norepinephrine
5-HT	5-hydroxytryptamine
ENT	ears, nose and throat
IV	intravenous
GC-MS	gas chromatography-mass spectrometry
IR	infra-red
CNS	central nervous system
MEA	microelectrode arrays
hDAT	human dopamine reuptake transporter
hNET	human norepinephrine reuptake transporter
SPE	solid-phase extraction
QuEChERS	quick (Qu), easy (E), cheap (Ch), effective (E), rugged (R) and safe (S)
LLE	liquid–liquid extraction
dSPE	dispersive solid phase extraction
ELISA	enzyme-linked immunosorbent assay
UHPLC	ultrahigh-performance liquid chromatography
TOF	time-of-flight
UV-Vis	ultraviolet-visible
PDA	photodiode array detector
HPLC	high-performance liquid chromatography
LC-HRMS	liquid chromatography–high-resolution mass spectrometry
CYP2D6	cytochrome P450 2D6
FMO3	flavin-containing monooxygenase 3
NAT1	*N*-acetyltransferase 1
NAT2	*N*-acetyltransferase 2

SGK1	serum/glucocorticoid regulated kinase 1
PER2	period circadian regulator 2
CB	cannabinoid receptor
EMCDDA	European monitoring centre for drugs and drug addiction
CBD	cannabidiol
CBN	cannabinol
GPCR	G protein-coupled receptor
CB1R	cannabinoid receptor type 1
CB2R	cannabinoid receptor type 2
pHLM	pooled human liver microsome assay
SARs	structure–activity relationships
AAI	aminoalkylindole
SCs	synthetic cannabinoids
UGT	UDP glucuronosyl transferase
HEK293	human embryonic kidney 293
HNK	hydroxy-norketamine
NPD	nitrogen–phosphorus detector
CIMS	chemical ionization mass spectrometry
HS-SPME	headspace-solid phase microextraction
AMT	alpha-methyltryptamine
DIPT	diisopropyltryptamine
MAO	monoamine oxidase
NSO	new synthetic opioids
LOD	limit of detection
DBZD	designer benzodiazepines
US-LDS-DLLME	ultra-assisted low-density solvent dispersive liquid–liquid microextraction
QSAR	quantitative structure–activity relationship
DFT	density functional theory
UPLC	ultra-performance liquid chromatography
DART	direct analysis in real time
TLC	thin layer chromatography
EIA	enzyme immunoassay
APCI	atmospheric pressure chemical ionization
NACE	nonaqueous capillary electrophoresis
UPC2 SFC-PDA	ultraperformance convergence chromatography supercritical fluid chromatography–photodiode array
NIRS	near-infrared spectroscopy
HPTLC	high-performance thin-layer chromatography
FID	flame-ionization detection
ECD	electrochemical detection
DESI-MS	desorption electrospray ionization mass spectrometry
HSCCC	high-speed counter-current chromatography
4-MMC	4-methylmethcathinone
MDPV	3,4-methylenedioxypyrovalerone
4-MEC	4-methylethcathinone
4-MePPP	4′-methyl-α-pyrrolidinopropiophenone
α-PVP	α-pyrrolidinopentiophenone
4-FMC	4-fluoromethcathinone
3-FMC	3-fluoromethcathinone
α-PBP	α-pyrrolidinobutiophenone
3-MMC	3-methylmethcathinone
MDMA	3,4-methylenedioxymethamphetamine
MDPBP	3′,4′-methylenedioxy-α-pyrrolidinobutyrophenone
3,4-DMMC	3,4-dimethylmethcathinone
PV9	1-phenyl-2-(pyrrolidin-1-yl)octan-1-one
IS	internal standard
TM5	transmembrane helix 5
TM6	transmembrane helix 6
r5-HT_{2A}R	rat HT_{2A} receptor
BZP	benzylpiperazine

1 Introduction

The number of health-related incidents caused by the use of illegal drugs is increasing rapidly, and so is the need for better understanding of their physiological effects and fast identification [1].

These substances can be grouped depending on the chemical structure into synthetic cannabinoids, synthetic cathinones, phenethylamines, arylcyclohexylamines, tryptamines, indolalkylamines, new synthetic opioids, piperazines and designer benzodiazepines [2,3], and on the basis of their origin on psychoactive drugs of natural origin and synthetic molecules. Previous studies have been limited to the analytical and toxicological data related to only some classes, and their representatives [4–30], reviews of the particular group [31–35], or particular topic [36], but failed to address all aspects: identification, quantification, synthesis, case reports, and statistics. The last few years have witnessed research on origin and the trafficking route for various psychoactive molecules (using both ^{13}C NMR spectrometry and ^{13}C, ^{15}N MS) [37], and the use of machine learning to predict the similarity of new psychoactive substances (NPS) with the classical NPS [38]. This article seeks to address the topic in the broadest spectrum available.

2 Psychoactive drugs of natural origin

Numerous plants possess psychoactive properties. *Areca catechu*, *Argyreia nervosa*, *Ayahuasca*, *Catha edulis*, *Ipomoea violacea*, *Mandragora officinarum*, *Mitragyna speciosa*, *Pausinystalia johimbe*, *Piper methisticum*, *Psilocybe*, *Rivea corymbosa*, *Salvia divinorum*, *Sceletium tortuosum*, *Lactuca virosa*, and *Lophophora williamsii* have been receiving much attention due to their common misuse [34]. Mainly found in Asia and South America, the misuse of these plants is underestimated due to religious and traditional practices [34]. *Catha edulis* (common name: khat; mainly in the USA and the Netherlands), *Mitragyna speciosa* (common name: kratom; mainly in Asia), and *Salvia divinorum* are monitored by the United Nation Office on Drugs and Crime [34].

2.1 *Areca catechu*

Areca catechu belongs to Arecaceae family, and it is a native palm tree in Sri Lanka and Malaysia, abounded in Asia and Africa and exported to USA and Europe by the Asian communities. The fruit of this plant (areca nut) is traditionally chewed and represents one of the most used drugs (after caffeine, ethanol, and nicotine) [32]. It is consumed either in combination with other substances ("betel quid") or alone giving the stimulation and relaxation during ceremonies and as a traditional remedy in China [39]. The psychoactive property of the plant is mainly caused due to the presence of arecoline, a GABA competitive inhibitor inducing agitation and euphoria [40–42]. The available data on the analytical determination of the active component are presented in Table 1.

There are no measures regarding the use of the *Areca catechu* or its active compounds in the EU and the USA [34].

2.2 *Argyreia nervosa, Ipomea violacea* and *Rivea corymbosa*

Argyreia nervosa (common names: Hawaiian Baby Woodrose, Adhoguda or Vidhara, Elephant Creeper, and Woolly Morning Glory), *Ipomea violacea* (common name: morning glory), and *Rivea corymbosa* are plants with characteristic pink flowers with the origin at the Indian subcontinent and transferred to Africa, Europe, and subtropical America [43]. The psychoactive alkaloids (isoergine and ergine) are mainly found in the plant seeds, and they show psychoactive effects quite similar to lysergic acid diethylamide (LSD), but not so intensive [3,44]. Similarly to ergot alkaloids, ergine is assumed to bind to D2-dopamine receptors [45]. The available data on the analytical determination of the active components are presented in Table 1.

There are specific national regulations regarding LSA in Italy and UK. In the USA, the LSA and its related products are controlled (Schedule III drug in the Controlled Substances Act) as a depressant, and LSA is also on the list of U.S. Code of Federal Regulations as a possible LSD forerunner, but the plant and the seeds can be bought without any problem [34].

2.3 *Banisteropsis caapi* and *Psychotria viridis*

Ayahuasca (Quechua word meaning "soul rope") is a brew characteristic for the South America used for religious and therapeutic purposes in Northwestern Amazonian countries for many centuries, and now by some religious sects (Santo Daime, Baraquinha; prepared from *Banisteropsis caapi* stems mixed with *P. viridis*, *Mimosa hostiles*, *Mimosa tenuiflorea*, *Anadenanthera* spp., and/or other plants with psychoactive compounds) [10,46]. The psychoactive compound found in Ayahuasca is DMT that behaves as $5HT_{A/2c}$ receptor agonist [47].

According to Hamill et al. [48], Ayahuasca has effect on the pupil size, body temperature, cardiovascular system, endocrine system, immune system but has shown no addiction potential. The most common side effects are agitation, hypertension, tachycardia, mydriasis, and vomiting.

The available data on the analytical determination of the active components are presented in Table 1.

There is a controversy about the control status of Ayahuasca because of its composition. Consumption of β-carbolines and *P. viridis* are not forbidden [34].

2.4 *Chata edulis*

Chata edulis (common name: khat) belongs to the Celestraceae family, and it is a native plant of Ethiopia, Arabian Peninsula, East Africa and used widely in Yemen [49]. Its use is forbidden in Denmark, Germany, France, Ireland, the United States, and Canada, while it is used as a

Table 1: Techniques used for the detection of new psychoactive substances of natural origin

Plant	The main detected compound	Biological matrices	Method used	References
Areca catechu	Arecoline	Plant material	HPLC	[41]
	Arecoline	Human plasma		[84–92]
		Saliva	GC	
		Hair	HPLC	
		Buccal cells	UPLC	
		Meconium	DART-MS/MS	
		Cord serum		
		Breast milk		
Argyreia nervosa, *Ipomea violacea*, and *Rivea corymbose*	LSA	Human blood urine	UPLC	[93,94]
	LSA	Plant material (seeds)	HPLC	[44,95–97]
			TLC	
		Capsules	GC	
Banisteropsis capii and *Psychotria viridis*		Plant material	HPLC	[98–106]
			GC	
	Harmine		GC	
	Harmaline		LC	
	DMT		DART-HRMS	
			HPLC	
	Harmine	Human urine	UHPLC	[98,107–111]
	Harmaline	Plasma	LC	
	DMT	Hair	HPLC	
			GC	
Chata edulis	Cathinone	Plant material (leaves and green)	HPLC	[112–114]
	Cathine		GC	
		Human urine	Immunoassay	[115–118]
	Cathinone	Hair	HPLC	
	Cathine	Blood	GC	
		Oral fluid		
Mandragora officinarum	Hyoscyamine	Human blood urine	GC	[119–125]
	Scopolamine	Plasma	HPLC	
		Plant material	EIA	
Mytragina speciosa	Mitragynine	Human blood	HPLC	[126–140]
	7-OH-mitragynine	Urine	1H-NMR	
		Rat plasma	13C-NMR	
			HPLC	
	Mitragynine	Plant material	HPLC, GC, NMR, HPLC, DART-HRMS, HPLC	[140–146]
	7-OH-mitragynine	Kratom products		
Pausinystalia johimbe	Yohimbine	Plant material	TLC, HPLC	[147–156]
	Yohimbine		HPLC	[157,158]
		Urine	NACE	
		Blood	GC	
		Plasma	UPLC	
			UHPLC	
Piper methysticum	Kawain	Kawa samples (powder or liquid)	UPLC	[159–181]
			HPLC	
		Plant material	UPC2 SFC detector, NIRS	
		Food supplement	HPTLC	
			GC	
			UHPLC	
			FTIR	
	Kawain	Human urine blood	GC	[182–186]
		Serum	LC	
		Hair	LC	

Table 1: continued

Plant	The main detected compound	Biological matrices	Method used	References
			GC	
Psylocibe spp.	Psilocin	Human urine	LC	[69,93,109,187–195]
	Psilocybin glucuronide	Plasma	HPLC	
		Serum		
	Psilocin	Plant material	LC	[174]
	Psilocybin	(sclerotia)		
Salvia divinorum	Salvinorin A	Plant material	TLC/DESI-MS	[196–203]
			GC	
			HPLC	
			TLC/GC	
			HPLC	
	Salvinorin A	Plasma	GC	[73,74,204]
		Urine		
		Saliva		
		Sweat		
Sceletium tortuosum	Mesembrine	Plant material	EC-MS	[205–208]
	Mesembrenone		UPLC	
			HSCCC	
	Mesembrine	Rat urine and plasma	GC	[209,210]
			LC	
	Mesembrenone	Human liver	LC, UHPLC	
Lactuca virosa	LC	Plant material	HPLC	[211–214]
	LCP		HPLC	
Lophophora williamsii	Mescaline	Plant material	HPLC	[215–218]
			LC	
			Ion-interaction HPLC	
			HPLC	

recreational and traditional habit in Ethiopia, Yemen, Israel, Somalia [49]. *S*-(−)-Cathinone is the main alkaloid in khat leaves [50]. Symptoms of psychosis and violent behaviors are widely displayed in khat chewers, particularly heavy consumers [34]. Identified toxic effects on the gastrointestinal system, respiratory, cardiovascular, endocrine, and genitourinary system cause increased blood pressure, tachycardia, constipation, insomnia, general malaise, headache, irritability, and impaired sexual potency are found in men [50–52].

The available data on the analytical determination of the active components are presented in Table 1.

2.5 *Mandragora officinarum*

Mandragora officinarum (common name: mandrake) is a native in the area of the eastern Mediterranean, but it is also abundant in the Middle East, southern Europe, northern Africa, and Himalayas [53]. It possesses aphrodisiac, healing, hallucinogenic, and poisonous properties [34].

The available data on the analytical determination of the active components are presented in Table 1.

In EU and USA, there are no legal measures regarding the use of the *Mandragora officinarum* or active compounds isolated from the plant [34].

2.6 *Mytragina speciosa*

Mytragina speciosa (common name: kratom) originated in South East Asia [47,54]. Fresh leaves are traditionally chewed, and the dried leaves can be smoked or chewed. Although the molecular structure of the active components (mitragynine, speciogynine, paynantheine, and speciociliatine) is different from opioids, they possess the affinity for opioid receptors leading to the analgesic effect (mytragynine to supraspinal μ-opioid receptors and δ-opioid receptors). The second mechanism of action is the inhibition of pain involving the release of the neurotransmitters by reversible blocking of the Ca^{2+} channels [55]. Gastrointestinal effects, anti-inflammatory properties, antidepressant activity, and antioxidant properties have also been published [56–58].

The available data on the analytical determination of the active components are presented in Table 1.

Mytragina speciosa and isolated active compounds are currently under control only in Latvia, Lithuania, Denmark, Romania, Poland, Sweden, and Italy. There is a narcotic law in Malaysia, Australia, Myanmar, and Thailand against kratom, and in New Zealand, Medicines Amendment Regulations control *Mytragina speciosa* and mitragynine. In the USA, the Drug Enforcement Administration has labelled kratom on its list as a "drug of concern."

2.7 *Pausinystalia johimbe*

Pausinystalia johimbe (from the family Rubiaceae) is native to tropical West Africa, and widely grows mainly in Cameroon [59]. Yohimbine, the major alkaloid found in the bark of this plant, is an α_2-adrenoreceptor blocker and a weak α_1-antagonist [60]. It is known to cause increase in heart rate, blood pressure, and plasma norepinephrine [60]. Yohimbine induces the increase in plasma NE levels by the increase in the rate of norepinephrine release from sympathetic nerves [60].

The use of yohimbe bark and its preparations is prohibited in foods or food supplements in UK, Ireland, the Netherlands, Belgium, Denmark, Czech Republic, Canada, Australia, and New Zealand, while in the USA, it is possible to possess it without license or prescription [34].

2.8 *Piper methysticum* Forst

Kava is a Pacific beverage traditionally used and made from the stems and roots of *Piper methysticum*, which belongs to the pepper family [61–63]. It is known to decrease anxiety and fatigue; it gives the user the sense of a sociable attitude, induces sleep, and relieves pain [51]. Six major kavalactones induce changes, interacting with GABA activity, inhibiting monoamine oxidase B, and reuptaking noradrenaline and dopamine [64].

The available data on the analytical determination of the active components are presented in Table 1.

Kava is legal in many countries; the use of the plant and the preparations containing kava lactones are legally regulated. The sale of *Piper methysticum* is controlled in France, Switzerland, and the Netherlands. Pharmaceutical preparations of the plant are prescription drugs in Germany. The UK government in 2002 clearly prohibited the sale, importation, and supply of kava-containing products. The possession of kava was strictly illegal in Poland until August 2018 [34].

2.9 *Psylocibe*

"Magic mushrooms" are the most common name for hallucinogenic fungi which contain psychoactive alkaloids psilocin and psilocybin. These alkaloids are two psychedelic substances with effects similar to LSD and mescaline [65–68].

The available data on the analytical determination of the active components are presented in Table 1.

Psylocin and psylocibine are considered as Schedule I drugs under the United Nations 1971 Convention on Psychotropic Substances and, therefore, mushroom containing them are not legal in the majority of worldwide countries. In the Netherlands, mushroom is illegal since December 2008. Mushroom and its active compounds are listed in Table 1 of the Republic Presidential Decree 309/90 and following updates in Italy [34].

2.10 *Salvia divinorum*

Salvia divinorum is an endemic plant in Mexico (the northeastern Sierra mazateca mountain) [69]. The chewing of fresh leaves or using it to make tea is known for centuries, while the dried leaves can be smoked or chewed [34].

Salvinorin A is the main active molecule of *Salvia divinorum*, and it is a potent hallucinogenic [70]. Different mechanism of action is shown by comparing it with classical hallucinogens, such as Δ9-tetrahydrocannabinol, LSD, or ketamine, due to no interaction with the 5-hydroxytryptamine receptor, *N*-methyl-D-aspartate receptor, and cannabinoid receptor [71,72].

The available data on the analytical determination of the active component are presented in Table 1.

Recently, both *Salvia divinorum* and salvinorin A have been brought under control in Belgium, Italy, Denmark, Lithuania, Latvia, Romania, Sweden, Japan, and Australia. *Salvia divinorum* has also been included recently in the list of "drugs of concern" by United States Drug Enforcement Administration. Germany, Croatia, Poland, and Spain put the control on the plant. *Salvia divinorum* is under medicines legislation in Finland, Estonia, and Norway. Without authorization under the

Natural Health Products Regulation, it is impossible to sell Salvia in Canada [34].

2.11 *Sceletium tortuosum*

Sceletium tortuosum (common names: channa, kanna, sceletium) is a plant that belongs to Mesembryanthemum family. It is grown and used (as quid) in southern Africa to elevate mood and to relieve thirst and hunger. On the websites, it is sold as capsules or tablets, and it is highly recommended for the treatment of depression and anxiety, to quit smoking, and among students during the intense study periods [34]. The psychoactivity is attributed to alkaloids, mainly mesembrine. It was isolated by Zwicky in 1914, and structurally solved in 1960 [73,74]. *In vitro* experiments reveal various pharmacological roles, such as an effective inhibition of 5-HT reuptake, while mesembrenone inhibits both phosphodiesterase type 4 isoenzyme and 5-HT reuptake [75,76].

Sceletium tortuosum induces lethargy, strong headaches, loss of appetite, and depression [77].

The available data on the analytical determination of the active components are presented in Table 1.

There are no measures regarding the use of the *S. tortuosum* or active compounds isolated from the plant in the EU as well as in the USA [34].

2.12 *Lactuca virosa*

Lactuca virosa (wild lettuce) is a plant which can be found in Europe and in Asia [78]. Various preparations of *Lactuca virosa* have been used traditionally as a natural diuretic, analgesic, antitussive, and sedative [78,79].

The available data on the analytical determination of the active components are presented in Table 1.

There are no measures regarding the use of the plant or its active compounds in the EU as well as in the USA [34].

2.13 *Lophophora williamsii*

The illicit administration of *Lophophora williamsii* is less common, and the licit consumption is associated with the rituals of religious nature connected to Native American Church. The pharmacodynamic mechanisms of action involve the interaction with 5-HT_{2A-C} receptors, inducing euphoria, hallucinations, depersonalization, and different psychoses [80]. However, it was shown to stimulate blood pressure, sleep, hunger, and thirst [81]. It was found that it contains different alkaloids, such as mescaline, pellotine, anhalonidine, lophophorin, anhalonin, anhalamin, *N*-methyl mescaline, *N*-acetyl mescaline, anhalidin, *O*-methylanhalonidine, and anhalin [82]. Mescaline was identified in 1896 and first synthesized in 1919 [80].

The available data on the analytical determination of mescaline are presented in Table 1.

Peyote is illegal in Brazil, Italy, France, and other countries, and it is not under control in Canada if it is not prepared for ingestion. The peyote use is only permitted when related to the Native American Church in the US legislation. The peyote cactus is not strictly prohibited or regulated in Mexico [83].

3 Synthetic molecules

3.1 Synthetic cathinones

Synthetic cathinones ("bath salts" in the USA and "plant food" or "research chemicals" in Europe) were synthesized in 1920s and were used for the treatment of patients with symptoms of Parkinson disease, obesity, and depression [219,220]. Lately, due to their psychoactive properties (empathy, euphoria, increased alertness, talkativeness, openness in communication, intensification of sensory experiences, reduced appetite, music sensitivity, increased sociability, insomnia, and capacity to work) [33], they were used as recreational drugs. However, synthetic cathinones possess somatic (cardiovascular system: hypovolemia, tachycardia, chest pain, hypertension, ST segment (the period when the myocardium maintains contraction to expel blood from the ventricles) alterations, myocarditis, cardiac arrest; central nervous system: insomnia, dizziness, headache, seizures, confusion altered mental status, tremor, confusion, dizziness, collapse, dystonia, hyperreflexia, drowsiness, myoclonus, paraesthesias; hematologic system: disseminated intravascular coagulation, anemia, thrombocytopenia; gastrointestinal and hepatic system: nausea, emesis, abnormal liver function tests, abdominal pain, liver failure; pulmonary system: tachypnea, shortness of breath, respiratory failure and arrest, respiratory acidosis; renal system: increased renal creatinine, kidney damage, hypo-

Table 2: Cathinone derivatives

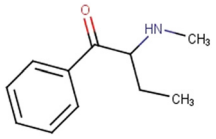
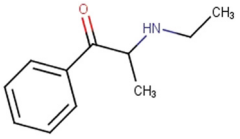
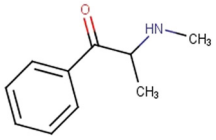
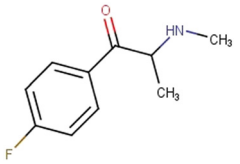
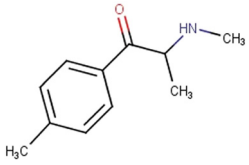
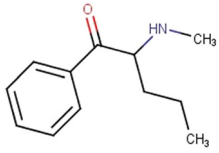
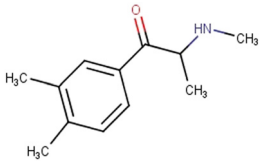
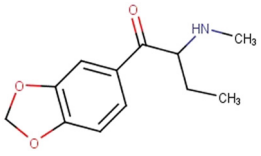
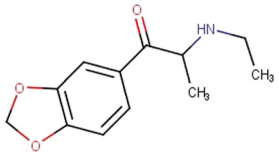
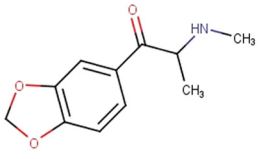
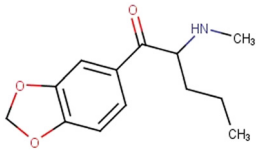
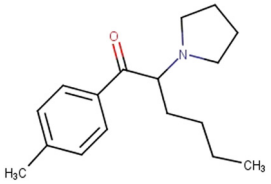
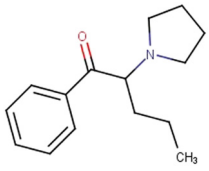
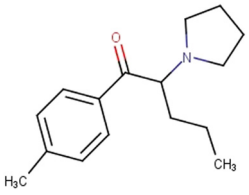
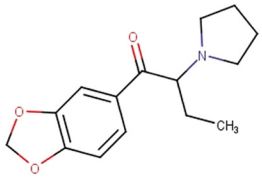
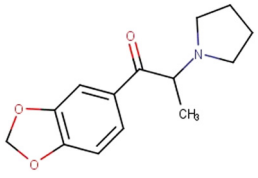
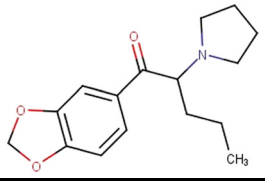
Chemical name	Common name	Chemical structure
[2-(*N*-Methylamino)butan-1-onyl]-benzene	Buphedrone, *α*-methylaminobutyrophenone	
[2-(*N*-Ethylamino)-propan-1-onyl]-benzene	Ethcathinone, ETCAT, *N*-ethylcathinone	
[2-(*N*-Methylamino)-propan-1-onyl]-benzene	Ephedrone, methcathinone, CAT, *α*-methylaminopropiophenone	
1-[2-(*N*-Methylamino)-propan-1-onyl]-4-fluorobenzene	Flephedrone, 4-FMC, 4-fluoromethcathinone	
1-[2-(*N*-Methylamino)-propan-1-onyl]-4-methylbenzene	Mephedrone, 4-MMC, 4-methylmethcathinone	
[2-(*N*-Methylamino)-pentan-1-onyl)]-benzene	Pentedrone, *α*-methylaminovalerophenone	
1-[2-(*N*-Methylamino)-propan-1-onyl]-3,4-dimethylbenzene	3,4-DMMC, 3,4-dimethylmethcathinone	
1-[2-(*N*-Methylamino)-butan-1-onyl]-(3,4-methylenedioxy)-benzene	Butylone, bk-MBDB, *β*-keto-methylbenzodioxolylbutanamine	
1-[2-(*N*-Ethylamino)-propan-1-onyl]-(3,4-methylenedioxy)-benzene	Ethylone, bk-MDEA, 3,4-methylenedioxy-*N*-ethylcathinone	
1-[2-(*N*-Methylamino)-propan-1-onyl]-(3,4-methylenedioxy)-benzene	Methylone, bk-MDMA, 3,4-methylenedioxy-*N*-methylcathinone	

Table 2: continued

Chemical name	Common name	Chemical structure
1-[2-(*N*-Methylamino)-pentan-1-onyl]-(3,4-methylenedioxy)-benzene	Pentylone, bk-MBDP	
1-[2-(Pyrrolidin-1-yl)-hexan-1-onyl]-4-methylbenzene	MPHP, 4-methyl-α-pyrrolidinohexanophenone	
1-[2-(Pyrrolidin-1-yl)-pentan-1-onyl]-benzene	α-PVP, α-pyrrolidinovalerophenone	
1-[2-Pyrrolidin-1-yl)-pentan-1-onyl]-4-methylbenzene	Pyrovalerone, 4-methyl-α-pyrrolidinovalerophenone	
1-[2-(Pyrrolidin-1-yl)-butan-1-onyl]-3,4-methylenedioxybenzene	MDPBP, 3,4-methylenedioxy-α-pyrrolidinobutiophenone	
1-[2-(Pyrrolidin-1-yl)-propan-1-onyl]-3,4-methylenedioxybenzene	MDPPP, 3,4-methylenedioxy-α-pyrrolidinopropiophenone	
1-[2-(Pyrrolidin-1-yl)-pentan-1-onyl]-3,4-methylenedioxybenzene	MDPV, 3,4-methylenedioxypyrovalerone	

natremia, hyperkalemia, acute renal failure, hyperuricemia; musculoskeletal system: elevated creatinine kinase, peripheral vasoconstriction, rhabdomyolysis; ophthalmic system: mydriasis, nystagmus, blurred vision; ENT: epistaxis, tongue disorder, oral and pharyngeal effects, bruxism, trismus; consequences of IV use: vein blockage, local infection, skin erosion, scab, lump, abscess, gangrenous tissue, blood clots, and large holes at overused injecting sites) and psychiatric adverse effects (aggression, anxiety, agitation, anorexia, paranoia, depersonalization, visual and auditory hallucinations, paranoid delusion, psychosis, depression, suicidal thoughts, anhedonia, self-harm, cognitive disorders: long-term cognitive impairments, place and time, loosening of association,

disorientation to names, addiction, withdrawal, and tolerance). Two pioneering representatives are methcathinone (CAT; in the 1930s and 1940s it was used in Russia as an antidepressant) and 4-methylmethcathinone ([4-MMC] mephedrone). The second most popular drug, methylone (3,4-methylenedioxy-*N*-methylcathinone), is usually combined with mephedrone (first synthesized in 1929), and 3,4-methylenedioxypyrovalerone (MDPV) is used due to reinforcing properties and the activation of brain rewarding circuitry [220–222]. Some new cathinones are used as substitute medications in therapy and treatment (e.g., bupropion (trade names Wellbutrin, Zyban) is prescribed as a smoking-cessation aid and for the treatment of depression) [223]. Pyrovalerone was intended to be a prescription drug to treat chronic fatigue, lethargy and obesity but was withdrawn from the legal market due to abuse in users [224–226]. 4-MEC, 4-MePPP, α-PVP, butylone (β-keto-*N*-methylbenzodioxolylbutanamine), pentedrone (α-methylamino-valerophenone), pentylone (β-keto-methylbenzodioxolylpentanamine), 3-FMC, 4-FMC, naphyrone (naphthylpyrovalerone), and α-PBP have no currently accepted medical use in treatment [227]. 3-MMC (metaphedrone) first appeared in Sweden in 2012 without any therapeutic use [228,229], and it is present on the illegal market as white powder or crystals, and according to users, it is less potent and intense than MDMA and 4-MMC [230]. Power et al. synthesized and analyzed 3-MMC using GC-MS, IR, and NMR in 2011 [231]. Christie et al. used Raman spectrometry to distinguish regioisomers, and it is fast and reliable, and, therefore, it can be used at airports [232].

Based on their chemical structures, cathinone derivatives are divided into four groups. The first group is a group of *N*-alkyl compounds, and compounds with a halogen or an alkyl substituent at any position of the aromatic ring: ephedrone, ethcathinone, flephedrone, mephedrone, buphedrone, and pentedrone (Table 2). The second group consists of compounds with substituents at any position of the aromatic ring as pentylone, methylone, and butylone, i.e., methylenedioxy-substituted compounds (Table 2). The third group is a group of natural cathinone analogs with *N*-pyrrolidinyl substituent. Finally, the fourth group consists of compounds that include both *N*-pyrrolidinyl and methylenedioxyl substituents.

Synthetic cathinones easily cross the blood–brain barrier (*in vitro* experiments) [221]. Also, β-keto-amphetamines cause CNS stimulating and sympathomimetic effects characterized by increased blood pressure, heart rate, mydriasis, and hyperthermia [50,233–240]. They are inhibitors of monoamine transporters. Their selectivity for serotonin receptors, norepinephrine transporter, and dopamine transporters is quite different. The mechanism of cathinone on neurotransmission consists of triggering of presynaptic dopamine release and reduction in the reuptake of dopamine which is similar to the mechanism of amphetamines. Interestingly, although they are binding to dopamine and serotonin receptors, the cathinone shows the highest affinity for norepinephrine receptors. It was also found that cathinone induces serotonin release and the inhibition of its reuptake [241].

Cathinones exist in two stereoisomeric forms, and each of them may possess different potency [222]. *S*-Enantiomers are present in khat. However, most ring-substituted psychoactive substances are present as racemic mixtures [242].

Regarding the potency of their inhibition of noradrenaline, dopamine, and serotonin reuptake and the ability to release these compounds, Simmler et al. [221] divided synthetic cathinones into three groups based on *in vitro* experiments:

1. Cathinones that act like cocaine and MDMA (*cocaine-MDMA-mixed cathinones*). The mode of action of compounds from this group consists of nonselective inhibition of monoamine reuptake, which exhibits better selectivity toward the dopamine transporter and promotion of serotonin release (similarity to MDMA). Methylone, mephedrone, ethylone, butylone, and naphyrone are cathinones from this group [221,233,235–237,239].
2. Cathinones that act like methamphetamine (*methamphetamine-like cathinones*). Their mechanism of action consists of the preferential reuptake inhibition of catecholamines and the release of dopamine. Flephedrone, methcathinone, and clephedrone (4-chloromethcathinone) are cathinones from this group [221,234].
3. Synthetic cathinones with pyrovalerone-based structures (*pyrovalerone cathinones*). The members of this group, MDPBP and MDPV, are very selective and potent inhibitors of the catecholamine reuptake with no neurotransmitter release effect [221,234].

The strength and the action of cathinone on the central nervous system are wide depending on numerous factors (e.g., age, sex, general health condition, degree of addiction, taking other psychoactive substances, use of medication, and use of alcohol) [35,243]. They all elicit psychomotor excitation, euphoria, feeling of increased empathy, increased interpersonal openness and self-assurance, and increased libido [50,220,222,244]. Overdose can result in numerous adverse effects (e.g., panic

and aggression, memory disturbances, hallucinations, memory loss, depression, and suicidal thoughts) [221]. The combination of measurements (MEA recordings and neuronal activity) with specific assays (monoamine reuptake transporter inhibition) shows as the primary mode of action the inhibition in hDAT and hNET of the investigated synthetic cathinones (4-MEC, 4-MMC, 3-MMC, pentedrone, methylone, α-PVP, and MDPV) [245].

Meyer et al. [237] proposed the mechanism of mephedrone metabolism involving *N*-demethylation to basic amines followed by the ketone functionality reduction, and methyl substituent hydroxylation of the aromatic ring (which enables its oxidation to the carboxylic acid). Uralets et al. [246] investigated the metabolites of 16 synthetic cathinones found in human urine upon their division into three groups according to their metabolization:

1. Buphedrone, mephedrone, 4-methylbuphedrone, 4-methylethcathinone, pentedrone, 3,4-DMMC, flephedrone, ethcathinone, and *N*-ethyl-buphedrone belong to the first group. Their metabolism follows the pattern of the synthetic cathinone precursors (i.e., cathinone and methcathinone). In urine of recreational drug users, metabolites were detected from the processes of β-ketone reduction and *N*-dealkylation (ephedrines and norephedrines as the main metabolites).
2. The second group includes 3,4-methylenedioxy-substituted cathinones (butylone, methylone, and ethylone), which are less prone to the β-keto reduction compared to the compounds of the first group. One of the explanations can be the existence of the 3,4-methylenedioxyl substituent in the aromatic ring. In the analyzed urine, the parent molecules were found [35].
3. α-Pyrrolidinophenones, such as α-PBP and α-PVP, which were initially thought not to further metabolize followed by the reduction of the ketone group or not to be changed in the urine, are the representatives of the third group [237,246,247]. Shima et al. [248] showed that the main α-pyrrolidinophenones metabolic pathways depend on the length of the parent molecule alkyl chain in humans. PV9 metabolism differs significantly from α-PVP and α-PBP, and it includes (1) reduction of the ketone group to the alcohol, (2) oxidation of the pyrrolidine ring to the pyrrolidone, (3) aliphatic oxidation of the terminal carbon atom to the carboxylate, (4) hydroxylation at the penultimate carbon atom to the alcohol, (5) oxidation to the ketone, and (6) combinations of the above steps [248].

Dickson et al. [249] described the preparation method of autopsy material for basic drug search: to 1 or 2 mL of the sample in the liquid state, a phosphate buffer (pH 6), and the internal standard (ethylmorphine or mepivacaine at the concentration of 0.5 mg/L) were added. The mixture was then ultrasonicated for 15 min and centrifuged. They were subsequently put on the top of the SPE cartridges (mixed-mode silica-based SPE), which were previously treated with 3 mL deionized water, 3 mL methanol, and 2 mL of the same phosphate buffer. Afterward, the cartridges were washed with 2 mL deionized water, 2 mL 20% aqueous acetonitrile, and 2 mL 0.1 M acetic acid. In the end, the cartridges were dried for 3 min in a vacuum, then in 3 mL methanol and 2 mL hexane, and again dried for 10 min in a vacuum. The elution afterward was performed with 3 mL dichloromethane/isopropanol/ammonium hydroxide (78:20:2, v/v/v), and after the evaporation of the solvent under nitrogen and the residue dissolution in 50 μL acetonitrile, the samples were made ready for the instrumental analysis. The introduction of QuEChERS technique into the toxicological analysis is mentioned in the majority of recent reports on the determination of synthetic cathinones from postmortem samples. Its use has several advantages compared to LLE and SPE, which are prone to the possible contamination of samples giving rise to the possibilities of inaccurate results and negative matrix results on analytical instruments. Usui et al. [250] used QuEChERS for the rapid extraction of psychoactive substances from human blood, demonstrating selectivity compared to SPE and simplicity as LLE. Also, QuEChERS is often cheaper and faster comparing to LLE and SPE. In the extraction/partitioning step, liquid samples are triple diluted with distilled water, followed by the placement in plastic test tubes containing 0.5 g of a commercial mixture (sodium acetate and magnesium sulfate), a stainless-steel bead, and 1 mL acetonitrile with IS. The content of the tube is vigorously mixed and centrifuged. In the case of acidic analytes, the acetonitrile layer can be used directly for the instrumental analysis. Contrary, for basic compounds, additional step, dSPE, must be performed, which requires 600 μL of acetonitrile supernatant into a test tube that contains a commercially obtained mixture of *N*-propylethylenediamine, then an amount of an end-capped octadecylsilane, and magnesium sulfate, for the purification. Afterward, the content of the test tube should be mixed and centrifuged, and the upper layer taken for the instrumental analysis.

The identification of cathinone derivatives always begins with the application screening methods that are not specific. In the case of powders, tablets, and capsules, colorimetric methods are used [251,253]. The most frequently used test for nitrogen-containing compounds

(used for the identification of amphetamine) is the Marquis reagent (formaldehyde and sulfuric acid). It does not give positive reaction for synthetic cathinones derived from mephedrone. Positive results are obtained with the compounds containing the methylenedioxyl substituent (e.g., MDPV). For MDPV, the additional test with the Chen reagent (copper monosulfide, acetic acid, and sodium hydroxide) can also be applied, and this test was considered as good for the ephedrine derivatives, too [252]. Colorimetric tests are good because they are fast and easy for the application. However, the disadvantage of this test is that it provides the identification only of the single structural part of a molecule, which is not sufficient for the identification of a compound. Immunoenzymatic assays are used for the screening of the biological material. The most commonly used assay is ELISA [253,254], but it was shown as nonspecific because of cross reactions (e.g., the reaction between MDPV and butylone) [254]. For synthetic cathinones, primarily GC [251,255–261] and LC were used coupled with different spectroscopic techniques [262–264]. CI is sometimes applied, but EI is mostly used [251,255–261]. GC-MS gave a simple mass spectrum in the positive ionization mode characterized by signals derived from iminium ions. Zuba [260] proposed a new method for the identification of synthetic cathinones using GC-EI-MS. Recently the distinguishing of regioisomers becomes possible due to the application of GC-EI-MS/MS [255]. LC-MS is used in the toxicological analysis because of its high selectivity and sensitivity [265–267]. UHPLC coupled with the time-of-flight mass spectrometry (TOF-MS) [268] and its quadrupole TOF (QTOF) is an extra technique for the high accuracy analysis of the active compounds in designer drugs [269]. The less-used detection system, for biological samples and drug products, is LC coupled with ultraviolet-visible (UV-Vis) spectroscopy using diode array or PDA detection which can be used only for screening [267,270–273]. Screen-printed graphite electrodes can be used for the detection of two metabolites of 4-MMC (4-methylcathinone and 4-methylephedrine) and, therefore, is a potential portable analytical sensor for the fast, cheap, reliable, and accessible detection and quantification of synthetic cathinone metabolites mainly for on-site analysis [274]. HPLC-MS/MS in combination with micro-solid-phase extraction as a preparation process using membrane-protected molecularly imprinted polymer (high selectivity) can also be used for synthetic cathinones monitoring in urine [275].

3.1.1 MDPV

The alkaloid cathinone is the main psychoactive compound of the khat plant (*Catha edulis*), which has been used as a stimulant in the Arabian Peninsula and parts of Africa for hundreds of years. Its psychoactive properties are known for centuries by inhabitants of East Africa and north-eastern parts of the Arabian Peninsula [235,276–278]. It was found that members of this class stimulate the release of dopamine and norepinephrine [279] and inhibit dopamine and norepinephrine transporters with a negligible effect on serotonin reuptake [280,281]. MDPV was first synthesized in 1969 and is structurally closely related to cathinone [282,283]. Also, it is a locomotor stimulant, approximately ten times more potent than cocaine [234,284].

Table 3: Blood–brain barrier permeability for selected psychoactive substances [221]

	P_e ratio				
	Apical to basolateral	Basolateral to apical	Permeability	Active transport[a]	ClogP[b]
MDMA	6.0 ± 0.56	7.4 ± 2.4	+	No	1.85
Mephedrone	14.0 ± 10.4	12.2 ± 6.1	++	No	1.67
Methylone	6.1 ± 2.8	5.3 ± 1.3	+	No	1.39
Methcathinone	5.9 ± 2.8	8.5 ± 3.2	+	No	1.19
Amphetamine	6.3 ± 3.7	5.2 ± 1.3	+	No	1.74
Methamphetamine	5.4 ± 1.1	6.4 ± 3.0	+	No	1.74
MDPV	37.2 ± 11.3	12.0 ± 11.2	++	Yes	3.80

Data are expressed as mean ± SD (n = 3–9).

P_e ratios shows the blood–brain permeability of the drug in relation to the extracellular marker Lucifer yellow (P_e = 1).

+, high permeability (P_e ratio > 3). ++, very high permeability (P_e ratio > 10).

[a] $P < 0.05$ significant difference between apical to basolateral compared with basolateral to apical transport indicating active transport.

[b] ClogP, prediction of partition coefficient (lipophilicity).

Mephedrone and MDPV show excellent blood–brain barrier permeability in an *in vitro* model (Table 3) [221].

Users of this and similar drugs have experienced euphoria, alertness, talkativeness, sexual arousal, and positivity 30–45 min after oral intake, which lasts 1–3 h. The side effects are numerous and consist of insomnia, anxiety, mydriasis, fatigue, agitation, aggression, panic, combative behavior, disorientation, memory loss, confusion, blackouts, excited delirium, myoclonus, paranoia, hallucinations, chest pain, increased suicidal intention, and hypertension. According to medical records, users of these drugs (including MDPV) are out of control and very violent [285].

MDPV shares structural similarities and pharmacodynamics with MDMA. Recreational use of more than one drug is quite common [286]. Frequently, NPS are often combined with other drugs, particularly ethanol [287–289]. The MDPV levels in 23 postmortem cases ranged 10–640 ng/mL in blood [285].

3.2 Phenethylamines

Phenethylamines are compounds which are relatives of amphetamines and MDMA. Their skeleton is an aromatic ring with two-carbon side-chains ending with an amine group (Figure 1) and can undergo two main changes: (1) substitution of the α-carbon by a methyl produces amphetamine derivatives (Figure 1) [290] and (2) substitution of the benzene cycle at positions 2 and 5 with methoxy groups and position 4 with a substituent on phenethylamine or amphetamine (Figure 1) [290–292]. Tetrahydrobenzodifuranyl and benzodifuranyl ("FLY") are analogs of these series [293]. NBOMe series, which consists of *N*-benzyl derivatives of the 2 C series (Figure 1) was recently made [294,295].

It has been demonstrated that ring substitutions increase the affinity of compounds for 5HT2a receptors [297]. The substitution of the aromatic ring with a methylenedioxy group at positions 3 and 4 gives MDMA and its derivatives. They belong to a new pharmacological class – entactogens.

MDMA switches on central α_{2A} adrenoceptors and peripheral α_1 adrenoceptors inducing vasoconstriction to restrict heat loss, and β_3 adrenoceptors in brown adipose tissue increasing the generation of heat. The hyperthermia happening in recreational users of MDMA can be fatal (the first investigations in 1998 [298]); furthermore, the literature data indicate that there are small chances that any pharmaceutical agent will be

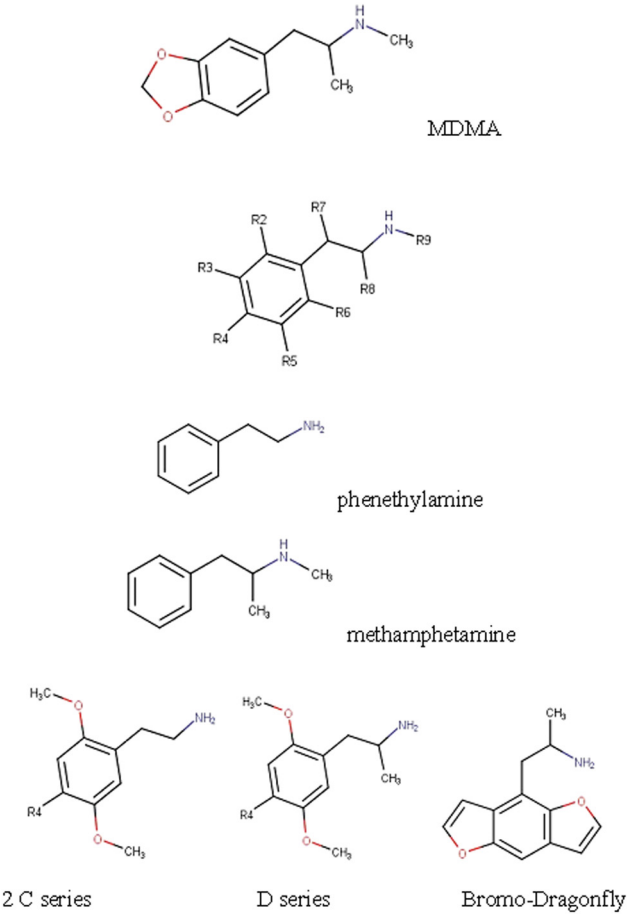


Figure 1: Chemical structures of some members of the phenethylamines family of drugs [296]. Reprinted from Drug and Alcohol Dependence, 154, Gael Le Roux, Chloe Bruneau, Benedicte Lelievre, Marie Bretaudeau Deguigne, Alain Turcant, Patrick Harry, David Boels, Recreational phenethylamine poisonings reported to a French poison control center, 46–53, 2015, with permission from Elsevier.

effective in reversing the hyperthermia [298]. Although it was found that hypothermia is the major effect when 10 mg kg^{-1} was injected in mice, hyperthermia followed by hypothermia is observed when doses of 30 mg kg^{-1} were applied [299]. The reason for that phenomenon may be the vasodilation of the tail veins [300]. Generally, MDMA and its derivatives do not cause hallucinations but promote the feeling of socialization in consumers [301–303].

Adverse reactions upon the consumption of phenethylamines are feelings of distress and anxiety, emotional disturbances, unpleasant hallucinations, tachycardia and hypertension, frequent agitation, tremors, and seizures [296]. It has been suggested that alcohol-induced effects are reduced by MDMA without any improvement in psychomotor performance. Effects of

MDMA was prolonged in combination with alcohol [304], but it reduces hyperthermia induced by MDMA [115]. Cannabis consumption combined with phenethylamines is frequent and recommended in Internet forums for avoiding "come down" (e.g., negative symptoms or aggressive behavior) [305].

Both *in vivo* and *in vitro* investigations of selected "FLY" analogs (2C-T-7-FLY, 2C-E-FLY, 2C-EF-FLY) using LC-HRMS/MS gave 32 metabolites with the major metabolic steps consisted of hydroxylation and *N*-acetylation; phase I was catalyzed by CYP2D6, 3A4, and FMO3 and *N*-acetylation using NAT1 and NAT2 [306]. LC-MS/MS methods for the thermally labile (25-NBOH drugs) were developed [307]. Pharmacokinetic profile of new amphetamines (1-(2,3,6,7-tetrahydrofuro[2,3-*f*][1]benzofuran-4-yl)propan-2-amine and 2-(2,3,6,7-tetrahydrofuro[2,3-*f*][1] benzofuran-4-yl)ethanamine) were investigated using LC-MS/MS [308]. Developed ELISA for the detection of 2C-B and similar hallucinogenic phenethylamines was confirmed as a good tool for screening before confirmation with UHPLC-MS-MS [309].

The fragmentations of NBOMe derivatives were analyzed using LC-QTOF/MS; the halogen-substituted methoxybenzylethanamine-type derivatives showed a characteristic product ion of a radical cation [14]. Fully validated LC-tandem mass spectrometry method was developed for the quantification of seven NBOMes (25B-, 25C-, 25D-, 25E-, 25G-, 25H-, and 25I-NBOMe) in blood, with the previous refrigeration of the whole blood (up to 90 days) or freezing of samples for longer storage [310].

3.2.1 25I-NBOMe

25I-NBOMe is a derivative of the 2C-X series of phenethylamines. NBOMe compounds are known as hallucinogens and stimulants, and potent agonists of the human $5HT_{2A}$ receptor [311].

Theoretical studies revealed expected interactions of partial agonists (hallucinogens like ergolines, phenylisopropylamines, and substituted tryptamines) with the $5HT_{2A}$ receptor (e.g., with a cluster of aromatic amino acids in TM5 and TM6, and serines in TM3 and TM5). The highly conserved $Asp155^{3.32}$ [312,313]; the serines $Ser159^{3.36}$, $Ser239^{5.43}$, and $Ser242^{5.46}$ (h5-HT_{2A}R, Ala242 in r5-HT_{2A}R) [314–316]; and the phenylalanines $Phe243^{5.47}$, $Phe244^{5.48}$, and $Phe340^{6.52}$ [317–319] are shown as important for efficacy and binding of agonists and partial agonists for $5HT_{2A}$R (superscripts show the generic numbering scheme of amino acids in TMs 1–7 proposed by Ballesteros and Weinstein [320]).

Dopamine level increased in mice after taking 25I-NBOMe, and the expression levels of SGK1 and PER2 changed [321].

3.2.2 MDMA

MDMA is the main constituent of the widely used recreational drug ecstasy [322]. It was first made in the lab in 1912 by Merck KGaA (Darmstadt, Germany) in the project aimed the identification of new hemostatic (blood-clotting) agents [322]. Its first major toxicological study in animals was performed in the 1950s at the University of Michigan in a classified USA Army contract [322]. In 1973, the results were declassified and made public by Hardman et al. [323]. In 1978, Alexander Shulgin together with David Nichols from Purdue University published the first report on the effects of MDMA in humans [324].

The positive effects of MDMA consumption include arousal, euphoria, increased sociability, enhanced mood, and heightened perceptions [322]. Adverse effects consist of headache, nausea, bruxism, tachycardia, and trismus [322]. The acute effects of MDMA are ascribed to increase the release and inhibit the reuptake of norepinephrine and serotonin with the possibility of the release of the neuropeptide oxytocin [322].

MDMA is a Schedule I compound by the Drug Enforcement Agency, but MDMA-assisted psychotherapy for patients with chronic, treatment-resistant posttraumatic stress disorder is currently under investigation [322].

3.3 Cannabinoids

Based on the origin, cannabinoids can be classified into (1) phytocannabinoids, (2) endocannabinoids, and (3) synthetic cannabinoids [325].

In the 1980s, cannabinoid receptors were found and labeled with CB and numbered according to their discovery by a subscript (CB_1 and CB_2). These receptors differ based on their predicted amino acid sequence, tissue distribution, and signaling mechanisms [326].

HPLC-UV approach was shown as the gold standard for the quantitation of the synthetic cannabinoids with highly conjugated chromophores [327]. Synthetic and natural cannabinoids were found in oral fluid using solid-phase microextraction coupled to gas chromatography/mass spectrometry [328].

Synthetic cannabinoids can be grouped based on their structures by the National Forensic Services:

naphthylindoles, phenylacetylindoles, benzoylindoles, cyclopropylindoles, aminocarbonylindazoles, adamantylindoles, adamantylindazoles, quinolinylindoles, CP-47,497 homologs, and cyclopropylthiazoles [1,329–331] (Figure 2).

The first synthetic cannabinoid was detected at the end of 2008, and since then, more than 130 synthetic cannabinoids have been registered at the EMCDDA [332].

3.3.1 Marijuana (cannabis)

Phytocannabinoids are present in significant quantities in plant cannabis [333]. The medicinal use of marijuana, a complex plant, for its analgesic, anticonvulsant, and anti-inflammatory properties is known [334]. The first medical data on this plant (the relief of cramps and pain) are coming from China around 5,000 years ago [335]. Few phytocannabinoids, especially CBD, has a beneficial effect in numerous pathological conditions (inflammation, cancer, addiction, and epilepsy) [336–339]. The various pharmacological properties of marijuana have inspired drug discovery programs intending to produce new cannabinoids with therapeutic potential.

However, a number of epidemiological research shows the connection between dose-related marijuana use and an increased risk of the development of symptoms of depression and anxiety [340]. Studies showed that the negative effect of cannabis is more pronounced in individuals with predispositions for psychosis and personality and psychosis susceptibility genes [340].

3.3.2 Synthetic cannabinoids

Synthetic cannabinoids are mimetic of Δ^9-tetrahydrocannabinol, the primary active substance in cannabis. Other cannabinoids present in cannabis are CBD and CBN [333,341]. They are full agonists of the CB1 receptor, a GPCR [342]. Several other receptors, ranging from other

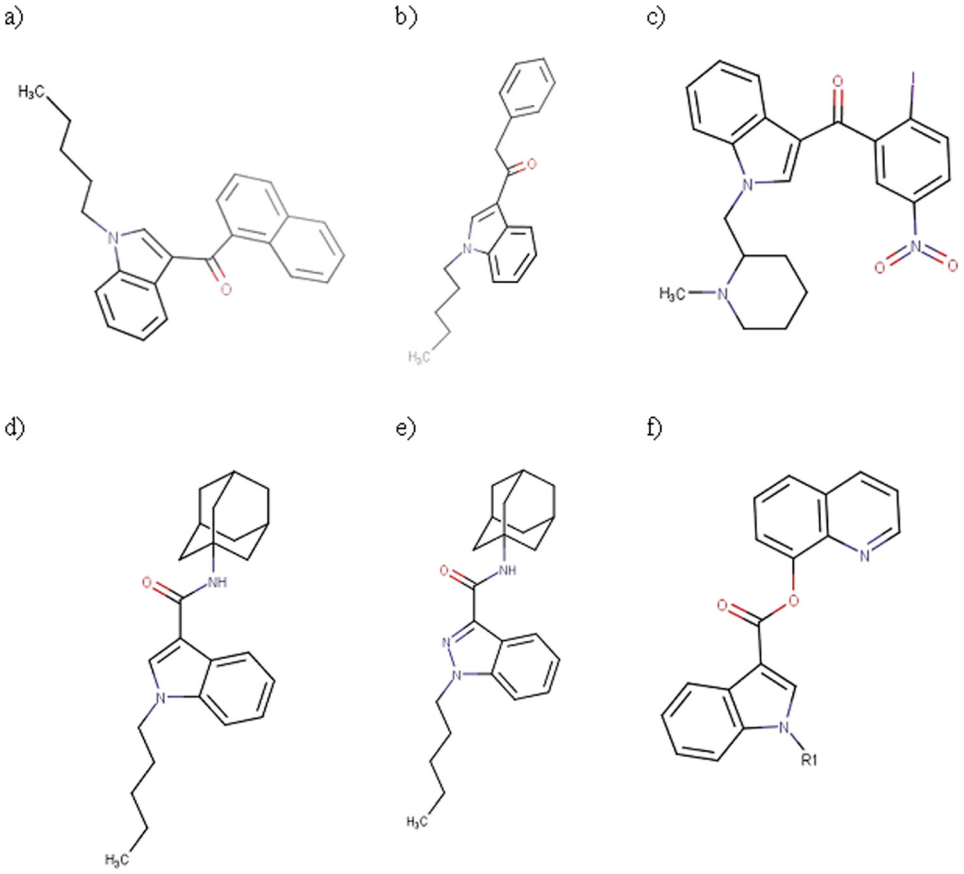


Figure 2: Sample structures for synthetic cannabinoids: (a) JWH-018, a simple naphthoylindole; (b) JWH-167, a simple phenylacetylindole; (c) AM-1241, a chemical from benzoylindole family; (d) APICA (2NE1, SDB-001), a drug from adamantylindole group; (e) APINACA (AKB48), a drug from adamantylindazoles family; (f) general structure for quinolinylindole.

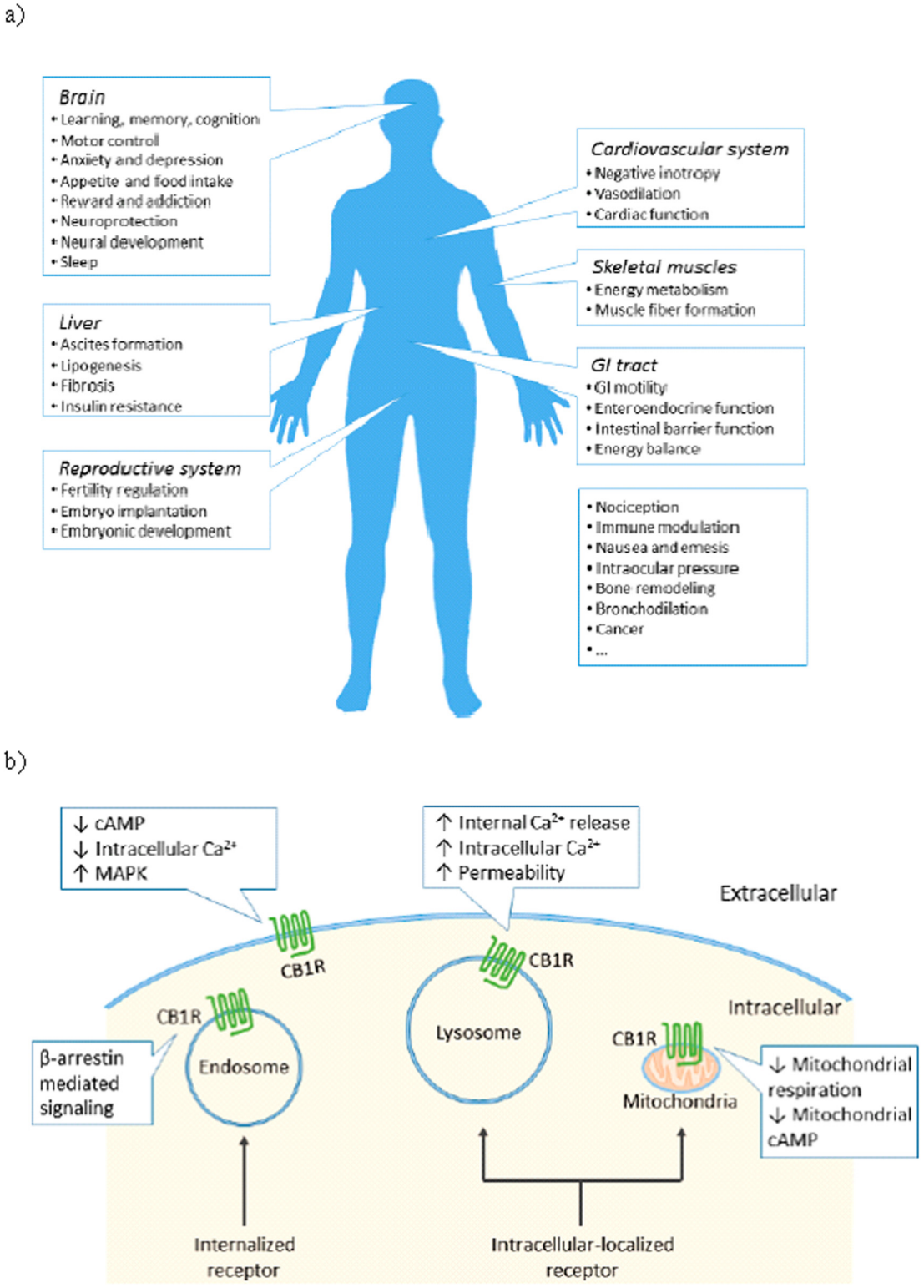


Figure 3: (a) The main localization sites and related functions of the CB1R in the human body; (b) subcellular localization of the CB1R [335].

GPCRs to ion channel and nuclear receptors, have been reported to have the interaction with cannabinoids [326,343]. The full-length CB1R dominates in the skeletal muscle and brain, whereas the CB1Rb shows high expression level in pancreatic islet cells and the liver [344] (Figure 3a). In human body, two isoforms of the CB2R

are as follows: the first is mainly expressed in testis and at lower levels in brain reward regions, whereas the second is predominantly expressed in the spleen and at the lower levels in the brain [345] (Figure 3a).

Similarly to other GPCRs, the CB1R is mainly localized in cell membrane. However, the predominant localization of CB1Rs is inside the cell, including transfected nonneuronal cells, cultured hippocampal neurons, and undifferentiated neuronal cells [346]. Intracellular CB1Rs are in acid-filled endo/lysosomes [347] (Figure 3b). Also, there is another subpopulation of CB1Rs expressed in mitochondria.

Synthetic cannabinoids have similar effects with the natural cannabinoids, including alteration in perception and mood, increased pulse, and xerostomia [348].

In vitro phase I of PX-1 (5F-APP-PICA) showed ten identified metabolites, which enable medical professionals and analytical scientists to detect PX-1 and make a prediction of the metabolites of synthetic cannabinoids with the similar structural pattern [349]. Synthetic cannabinoids with an alkene functional group at the alkyl side chain, chosen for *in vitro* and *in vivo* investigations (MDMB-4en-PINACA, methyl (*S*)-3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1*H*-indazole-3-carboxamido)butanoate) show a total of 32 metabolites (11 in hepatocyte samples, 31 in human liver microsomes, 1 in blood and 2 in urine), and the main metabolic pathway happens through the terminal alkene group of the pentenyl side chain consisting of dihydrodiol formation (*via* epoxidation probably) [350]. It was found that the major hydrolysis metabolites of ADB-CHMICA, 5F-AB-PINACA, ADB-FUBICA, ADB-CHMINACA, and their ethylester and methyl-derivatives do not induce any CB_1 activation at concentrations lower than 1 μM [351]. On the contrary, metabolites of 5F-ADB-PINACA, AB-CHMINACA, and ADB-FUBINACA show activity, but it is significantly reduced compared to the parent compounds (EC_{50} > 100 nM) [351]. 5F-CUMYL-P7AICA metabolites were identified in three urine samples, where the major biotransformation steps in humans were oxidative defluorination followed by carboxylation and monohydroxylation followed by sulfation and glucuronidation [264]. Metabolism of the new synthetic cannabinoid 7′N-5F-ADB in human, rat, and pooled human S9 was studied by means of hyphenated high-resolution mass spectrometry [352]. UHPLC-QTOF-MS was used for screening, quantification, and confirmation of synthetic cannabinoid (AB-FUBINACA, AB-CHMINACA, AB-PINACA, AM-2201, 5F-AKB48, AKB48, JWH-018, BB-22, JWH-081, JWH-073, JWH-122, JWH-203, JWH-250, 5F-PB-22, RCS-4, PB-22, THJ-2201, and UR-144) metabolites in urine [353]. Incubation of APP-CHMINACA with human liver macrosomes, followed by analysis with HRMS gave 12 metabolites with the predominant biotransformation in the form of hydrolysis of the distal amide group and hydroxylation of the cyclohexylmethyl substituent [354]. Analysis of pHLM and urine samples revealed that in case of 5F-AB-P7AICA the main metabolites were generated by amide hydrolysis, hydroxylation, and hydrolytic defluorination [355].

Huffman and colleagues at Clemson University extensively explored SARs within the AAI class of SCs, resulting in highly simplified analogs exemplified by JWH-018, which shows high affinity for CB_1 receptor (K_i = 9.0 nM) [356–359]. Auwarter et al. identified JWH-018 and the *n*-octyl homolog of CP 47497 as the psychoactive components of "Spice" [360].

3.3.2.1 JWHs

The first "JWH" compounds were made by Huffman et al. [357] researching the effects of JWHs on CB1 and CB2 receptors. They reported higher affinities than those reported for cannabis [359]. JWH-018 (1-alkyl-3-(1-naphthoyl) indole) was detected for the first time in 2008 in "Spice" products [360]. JWH-018 metabolite exerts higher toxicity compared to the parent drug, suggesting a non-CB1 receptor-mediated toxicological mechanism [361]. The first generation of JWHs consists of JWH-073, JWH-018, JWH-250, and CP 47,497. The synthesis of these drugs is straightforward, so it has been continued with second generation (RCS-4, JWH-122, and AM2201) [362].

Various JWHs were detected in seized materials [363–367], such as oral fluid [368], hair [369], serum, or whole blood [370–374]. *In vivo* studies consisted of the investigation of phases I and II metabolites in the rat urine after exposure and in human urine postadministration [375–383]. It was shown that hydroxylation and carboxylation are typical phase I biotransformations *prior to* conjugation [384]. JWH-018 is metabolized through phases I and II enzymes [385]. There are indications that CYP1A2 and CYP2C9 catalyze JWH-018 oxidation [386,387], while hepatic UGT1A9, UGT1A1, and UGT2B7 and extra-hepatic UGT1A10 are the enzymes that perform the catalysis of the conjugation of glucuronic acid to phase I JWH-018 metabolites [386]. JWH-018 hydroxylated metabolites bind to CB1 with more "love" than Δ^9 THC [388,389], and JWH-018 phase I metabolites also like CB2 receptor [389].

Investigations of toxicological profiles of synthetic cannabinoids have shown cannabinoid receptor independent and dependent cytotoxic effects on cell lines [390–392].

Koller et al. [391] found that JWH-018 induces damage to the cell membranes of buccal (TR146)- and breast (MCF-7)-derived cells at concentrations of ≥75–100 μM. JWH-018 *N*-(3-hydroxypentyl) phase I metabolite is toxic for HEK293T and SH-SY5Y cell lines contrary to its parent compound. JWH-018 metabolite causes mitochondrial damage and membrane disruption on both cell lines [361].

Different spectrometric techniques were used for the identification: GC-MS [236,364,377], GC-MS/MS [382], LC-MS [364], and LC-MS/MS [371,372,374–380,382,383,385]. Shanks et al. (2012) [373] developed the method for the analysis of the concentrations of JWH-018 and JWH-073 in human blood using UPLC-MS-MS. Concentrations ranged from 0.1 to 199 ng/mL for JWH-018, and 0.1–68.3 ng/mL for JWH-073 in postmortem forensic cases.

3.4 Arylcyclohexylamines

3.4.1 Ketamine and norketamine

Ketamine is a medical anesthetic agent used in veterinary medicine and also in humans [393,394] and pediatric practice [395]. Arylcyclo-alkylamine skeleton produces hallucinogenic effects [396].

Ketamine biotransformation mechanism of ketamine was established by Chang and Glazko (1972) [397]. In phase I, the ketamine oxidation process occurred (heteroatom demethylation), giving norketamine, followed by a hydroxylation process (here the product is HNK); and in phase II the biotransformation reaction, it undergoes glucuronidation and conjugation with glutathione and amino acid.

For the detection of ketamine, GC/NPD [398], GC/MS (first derivatized with heptafluorobutyric anhydride) [399], and GC/CIMS [400] were used. The same derivatization procedure was used for HS-SPME-GC/MS [401], LC/UV [402,403], and LC/MS single mass, tandem mass [404].

3.4.2 BZP

Stimulant properties of BZP, a piperazine derivative, are similar to those produced by amphetamine but less potent [405]. It is listed as Schedule I drug in the USA and Schedule III in Canada but banned in all Australian states, New Zealand, and Japan [406].

It has many adverse effects, such as palpitations, agitation, anxiety, confusion, dizziness, tremor, headache, urine retention, insomnia, and vomiting [407].

3.5 Tryptamines

Numerous biologically active derivatives contain the tryptamine nucleus as a building block, such as neurotransmitter serotonin or antimigraine drugs of the tryptan series. *N*,*N*-Dialkylation on nitrogen side chain may result in derivatives with psychoactive and hallucinogenic properties acting primarily as agonists of the 5-HT_{2A} receptor [408]. The story of synthetic tryptamines started with LSD in mid-1900s, with AMT 5-MeO-DMT (5-methoxy-*N*,*N*-dimethyltryptamine) and 5-MeO-DIPT as the next-generation designer drugs to replace LSD [408].

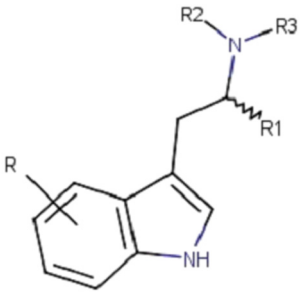
Key properties in the interpretation of mass spectra of this class of illegal drugs include the formation of iminium ion $C_nH_{2n+2}N^+$ in substituted $CH_2{=}N^+(R^1R^2)$ species. Soft-ionization techniques, such as electrospray, are used to give strong [3-vinylindole]$^+$-type species, reflecting the extent of the substitution on the indole ring [409].

Figure 4 represents a generalized tryptamine structure. Psychoactivity is highly affected by the substitution in positions 4 and 5 of the indole ring and the alkylation of the side-chain nitrogen and the side-chain carbon [410]. Interestingly, numerous naturally occurring psychoactive tryptamines are *N*,*N*-dimethylated derivatives: DMT, psilocybin (found in many mushroom species [411]), psilocin (4-OH-DMT), and 5-methoxy- and 5-hydroxy-DMT (bufotenin).

The pharmacology of tryptamine derivatives is complex, but it seems that 5-$HT_{1A\ \&\ 2A}$ receptor subtypes are involved [412–414]. It was found that DMT also serves as an agonist at the sigma-1 receptor [415]. Szara showed that DMT induces spatial distortions, visual hallucinations, speech disturbance, and euphoria when it is used intramuscularly in humans [416]. Numerous *N*,*N*-dialkylated tryptamines were discovered to be substrates at the vesicle monoamine transporter and the plasma membrane serotonin transporter [417].

LSD was synthesized in 1938 by Hofmann, and hallucinogenic properties were determined a few years later [418,419]. AMT was developed in the Soviet Union as an antidepressant under the name of Indopan in the 1960s. Although today it does not have any therapeutic applicability, its popularity as a "designer drug" increased in 1990s [420]. Tryptamine derivatives can be found as a free base or salt, tablets, or powders [421,422].

An increased impulsiveness and abnormal behaviors occurred after taking tryptamine. The study on rats was performed regarding the effect on body temperature. During the period of the administration, they showed hypothermia, followed by hyperthermia [408].



Serotonin: R=5-OH; R1=R2=R3=H

DMT: R=R1=H; R2=R3=CH_3

Psilocybin: R=4-OPO_3H_2; R1=H; R2=R3=CH_3

Psilocin: R=4-OH; R1=H; R2=R3=CH_3

5-MeO-DMT: R=5-OCH_3; R1=H; R2=R3=CH_3

5-OH-DMT: R=5-OH; R1=H; R2=R3=CH_3

Sumatriptan: R=5-$CH_2SO_2NHCH_3$; R1=H; R2=R3=CH_3

5,6-Br2-DMT: R=5,6-Br_2; R_1=H; R_2=R3=CH_3

5-Br-DMT: R=5-Br; R1=H; R2=R3=CH_3

5-MeO-DIPT: R=5-OCH_3; R1=H; R2=R3=$CH(CH_3)_2$

Figure 4: Generalized structure of a tryptamine derivative [409]. Reprinted from TrAC Trends in Analytical Chemistry, 29, Claudia P. B. Martins, Sally Freeman, John F. Alder, Torsten Passie, Simon D. Brandt, Profiling psychoactive tryptamine-drug synthesis by focusing on detection using mass spectrometry, 285–296, 2010, with permission from Elsevier.

DMT metabolic pathway in humans is sketched in Figure 5. It is inactivated by MAO enzymes in gut and liver.

The analysis of urine from LSD shows five metabolites: 2-oxo-LSD, 2-oxo-3-hydroxy-LSD, *N*-desmethyl-LSD, 13- and 14-hydroxy-LSD glucuronides [423–425]. It is suggested that 2-oxo-3-hydroxy-LSD could be made through dehydrogenation of the 2,3-dihydroxy-LSD intermediate, which is probably formed from LSD 2,3-epoxide [408]. Due to the fact that urine samples contain the parent compounds in small quantities or may not even be excreted, it is better to investigate the metabolites of NPS using pooled human liver S9 fraction. Such analysis of nine LSD derivatives (1-acetyl-LSD (ALD-52), 1-butyryl-LSD (1B-LSD), 1-propionyl-LSD (1P-LSD), N^6-ethyl-nor-LSD (ETH-LAD), N^6-allyl-nor-LSD (AL-LAD), 1-propionyl-N^6-ethyl-nor-LSD (1P-ETH-LAD), *N*-ethyl-*N*-cyclopropyl lysergamide (ECPLA), lysergic acid morpholide (LSM-775), and (2′*S*, 4′*S*)-lysergic acid 2,4-dimethylazetidide (LSZ)) enables the identification of monooxygenase enzymes involved in the initial metabolic steps [426]. It was found that 1-acyl-substitution reduces the affinity of LSD for the majority of monoamine receptors (including 5-HT_{2A} sites) [427]. 1P-LSD, ALD-52, and 1B-LSD have weak efficiency as antagonists in Ca^{2+} mobilization assays [427].

3.6 New synthetic opioids

NSO can be divided into two groups: (1) pharmaceutical (e.g., sufentanyl, fentanyl, remifentanyl, carfentanyl, and alfentanyl) and (2) nonpharmaceutical fentanyls (e.g., ocfentanyl and butylfentanyl). A new generation of NSOs, with structures different from fentanyls, appeared on the drug market in 2010: MT-45 (piperazine analogue), AH-7921 (benzamide analogue), isotonitazene, and U-47,700 (isomer of AH-7921) (Figure 6). They are characterized by different characteristics, such as availability on the Internet, purity, low price, legality, and lack of detection in laboratory tests [419]. NSOs can be found in tablet, powder, or liquid forms [428].

Their number is increasing. The synthetic opioid U-47700, μ-opioid receptor agonist, emerged on the illicit drug market, and it is sold as a "research chemical" with a potency of approximately 7.5 times that of morphine [418]. Its structure is similar to the synthetic opioid AH-7921.

Isotonitazene (*N*,*N*-diethyl-2-[5-nitro-2-({4-[(propan-2-yl)oxy]phenyl}methyl)-1*H*-benzimidazol-1-yl]ethan-1-amine) was identified recently using GC-MS and LC-QTOF-MS (m/z = 411.2398) with the confirmation of the region – isomer with ^{1}H and ^{13}C NMR [429]. Assessment of the *in vitro* biological activity at the μ-opioid receptor showed its high potency (EC_{50} = 11.1 nM) and efficacy (E_{max} 180% of hydromorphone) [429]. *In vivo* experiments show four metabolites identified using LC-QTOF-MS: *N*- and *O*-dealkylation products (*N*-desethyl-isotonitazene and *N*-desethyl-*O*-desalkyl-isotonitazene) were determined as urinary biomarkers, while 5-amino-isotonitazene was found in the majority of the investigated blood samples [430].

Potential use of NSO causes side effects, such as sedation, miosis, hypothermia, respiratory depression, inhibition of gastrointestinal propulsion, death (overdose) [431]. Sometimes reagents used in the synthesis can cause symptoms like discoloration of the nails, loss and depigmentation of hair, extensive folliculitis and dermatitis, bilateral hearing loss, elevated liver enzymes, and eye irritation followed by bilateral secondary cataracts requiring surgery, after the administration of MT-45 [432,433].

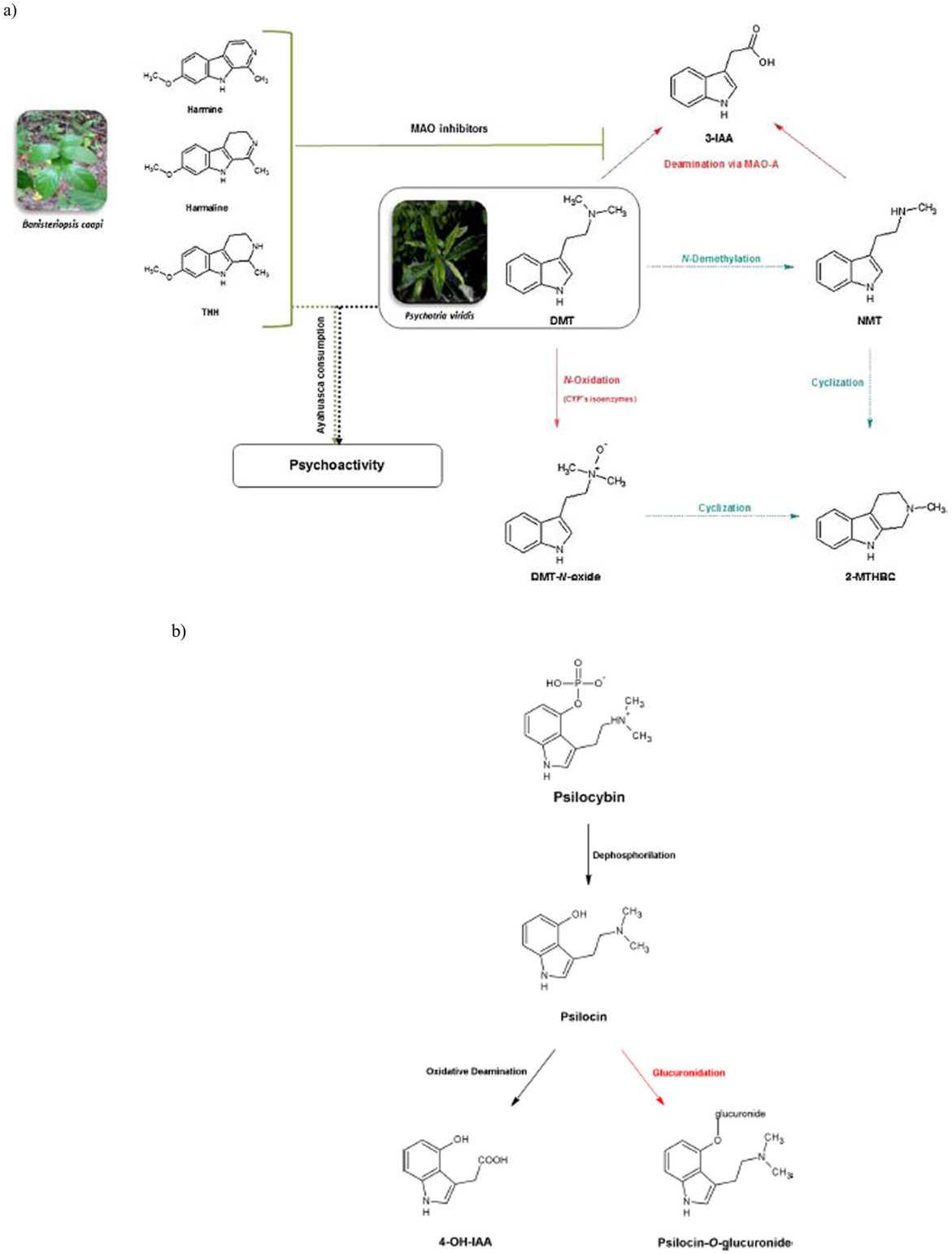


Figure 5: (a) Minor (blue arrows) and major (red arrows) metabolic pathways for DMT in humans; (b) metabolic pathways for psilocybin in humans [408].

As a pharmaceutical medicine, fentanyl is used in anaesthesia and for the management of severe pain. In anaesthesia, sufentanyl, remifentanil, and alfentanil can also be used. On the other hand, carfentanyl (at the moment the most powerful synthetic opioid-10,000 times more potent than fentanyl) is used in veterinary medicine

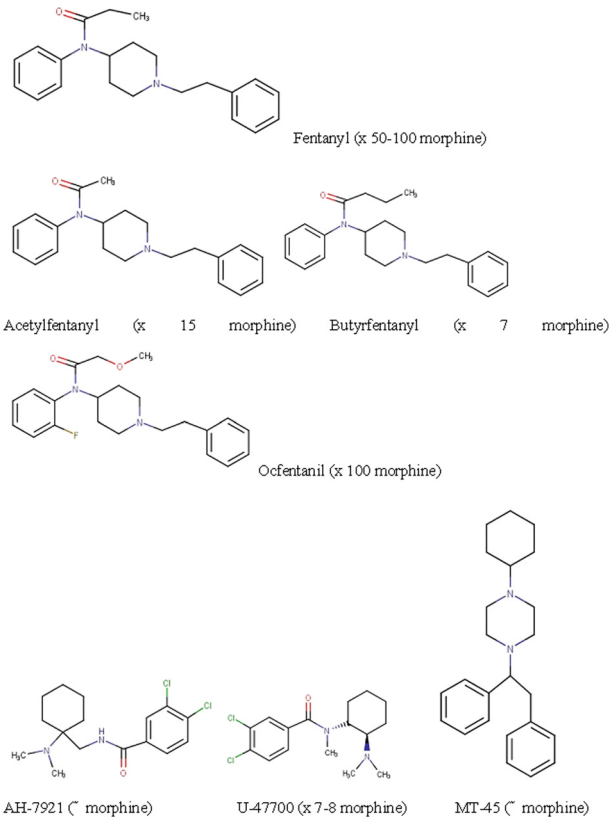


Figure 6: Structures and potencies of NSOs [428]

(trade name Wildnil®). However, it was most likely used to free hostages in Moscow by the government [434]. Pharmaceutical forms include lozenges, transdermal patch, sublingual tablets, and solutions for the infusion [435].

Up to 17 opioid receptors have been reported, but three classes are the most important in humans: μ, κ, and δ [436]. Fentanyl, made in 1959 by Jansen, is a complete μ receptor agonist. Metabolism of this drug, mediated by the CYP450 isoenzyme system, makes in active norfentanyl [437]. Carfentanyl and its metabolites can be detected in urine (LOD is 0.20 ng/mL for carfentanyl and LOD for carfentanyl metabolite is 0.01 ng/mL) [438]. Carfentanyl amides were found as potent compounds with less hazardous side effects associated with traditional opioids [439]. MT-45 acts on opioid (δ and κ) and nonopioid receptors. Its mechanism is not well investigated yet, and it can be responsible for special reported effects (e.g., profound loss of consciousness and ototoxicity) [440]. The psychiatric effects of opioids are related to the localization of receptors in the central nervous system [441]. κ receptors contribute to dysphoria, and they are present in the brain stem, spinal cord, and in the limbic and other diencephalic areas. Euphoria is connected to μ-opioid agonist effect in the medial thalamus and brain stem. The duration varies depending on the drug and its half-life (1–8 h). The psychiatric effects from opioid abuse are similar to those of heroin: a sense of well-being, relaxation, and euphoria, followed by a peaceful, dream-like state [428]. Naloxone is a short-acting semisynthetic competitive opioid receptor antagonist with the highest activity for the μ receptor and can be administered by intramuscular, intravenous, subcutaneous, and intranasal routes [428].

Routine urine drug tests cannot detect them yet. They show extreme potency, and very small quantities are enough to obtain a result [428]. The fragmentation pattern of frequently used NSO (fentanyl derivatives, AH series opioids, 4U series opioids, 4W series opioids, and MT-45) was investigated with the aim to be applied to a non-targeted screening workflow [442]. Metabolic fate of three NSOs (*trans*-4-bromo-*N*-[2-(dimethylamino)cyclohexyl]-*N*-methyl-benzamide (U-47931E), *trans*-3,4-dichloro-*N*-[2-(dimethylamino)cyclohexyl]-*N*-methyl-benzenacetamide (U-51754), and 2-methoxy-*N*-phenyl-*N*-[1-(2-phenylethyl) piperidin-4-yl]acetamide (methoxyacetylfentanyl)) was found using high-resolution mass spectrometry after pooled human S9 fraction incubation and in the urine of rats after oral intake, and the following main reactions occurred: (1) demethylation of the amine moiety for U-51754 and U-47931E, (2) *N*-hydroxylation of the hexyl ring, (3) combinations of *O*-demethylation, *N*-dealkylation, and hydroxylation at the alkyl part for methoxyacetylfentanyl [443]. Miniaturized ion mobility spectrometer with a dual-compression tristate ion shutter for on-site rapid screening of fentanyl drug mixtures was used [444]. Raman spectroscopy can also be used to distinguish fentanyls from morphines [445]. Analytical toxicology and toxicokinetic studies of the new synthetic opioids cyclopentanoyl-fentanyl (CP-F) and tetrahydrofuranyl-fentanyl (THF-F) revealed 12 phase I metabolites of CP-F and 13 of THF-F, among them 9 metabolites were described for the first time, with the *N*-dealkylations, hydroxylations, and dihydroxylations as the main metabolic reactions using LC-HRMS/MS [446]. Three fluorofentanyl isomers with the incubation with pooled human hepatocytes give as the major metabolite *N*-dealkylation product norfluorofentanyl, with 14 different metabolites for each fluorofentanyl isomer [447].

3.6.1 AH-7921

AH-7921 was reported first by EMCDDA in 2012. It was detected in synthetic cannabinoid products as well [1].

Table 4: Chemical structures and names of designer benzodiazepines [455]

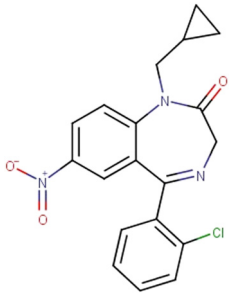
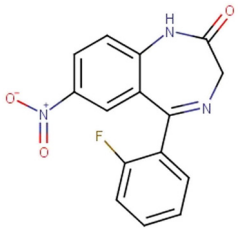
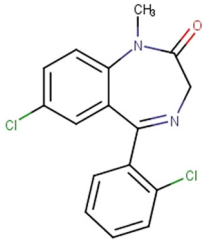
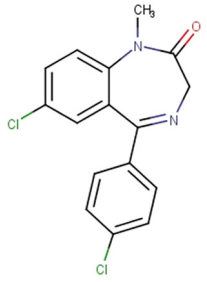
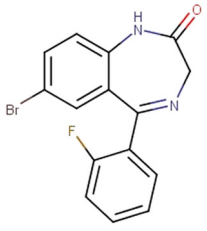
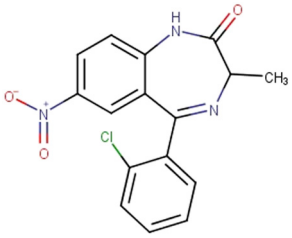
Chemical structure	Names
	**Cloniprazepam** (5-(2-chlorophenyl)-1-(cyclopropylmethyl)-7-nitro-1,3-dihydro-2*H*-benzo[*e*][1,4] diazepin-2-one
	**Desmethylflunitrazepam** or **norflunitrazepam** or **Ro-4435** or **fonazepam** (5-(2-fluorophenyl)-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one)
	**Diclazepam** or **2-Chlorodiazepam** (7-chloro-5-(2-chlorophenyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
	**4′-Chlorodiazepam** or **Ro5-4864** (7-chloro-5-(4-chlorophenyl)-1-methyl-3*H*-1,4-benzodiazepin-2-one
	**Flubromazepam** (7-bromo-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
	**Meclonazepam** or (***S***)-**3-methylclonazepam** (3*S*)-5-(2-chlorophenyl)-3-methyl-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one

Table 4: continued

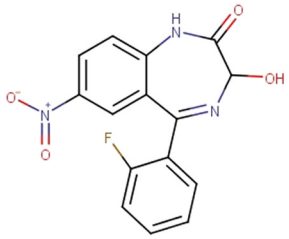
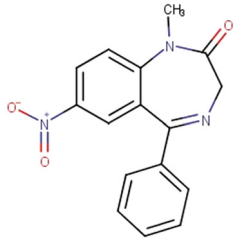
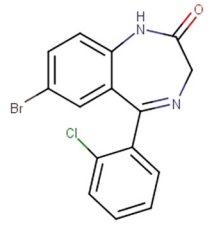
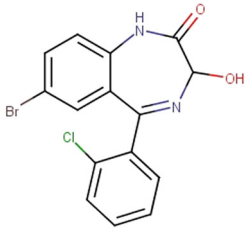
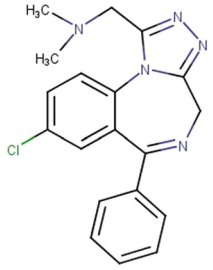
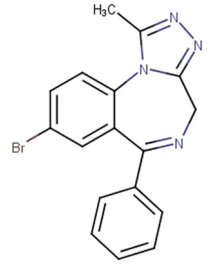
Chemical structure	Names
	**Nifoxipam** or **3-hydroxydesmethylflunitrazepam** (5-(2-fluorophenyl)-3-hydroxy-7-nitro-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one
	**Nitemazepam** (3-hydroxy-1-methyl-7-nitro-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-2-one
	**Phenazepam** (7-bromo-5-(2-chlorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one)
	**3-Hydroxyphenazepam** (7-bromo-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
	**Adinazolam** or **Deracyn®** or **Adinazolamum** (1-(8-chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine-1-yl)-*N*,*N*-dimethylmethanamine
	**Bromazolam** (8-bromo-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine

Table 4: continued

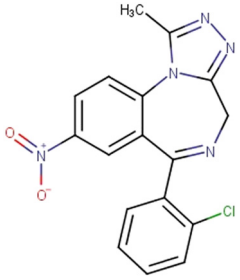
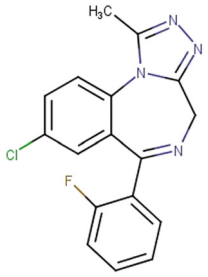
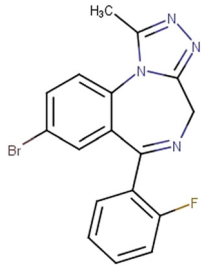
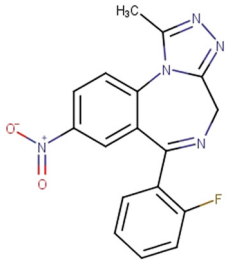
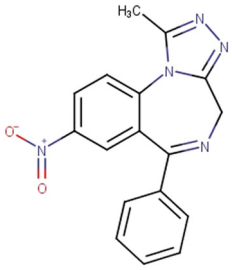
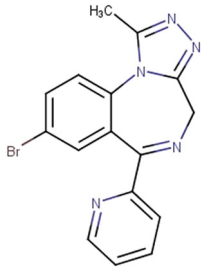
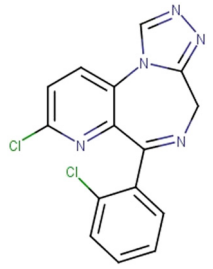
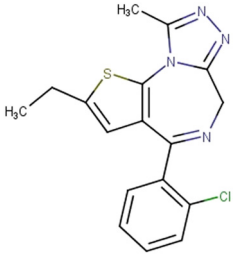
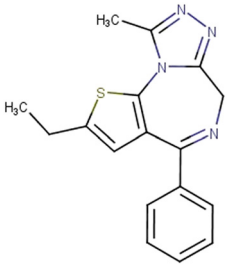
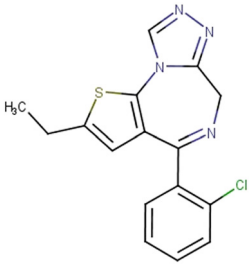
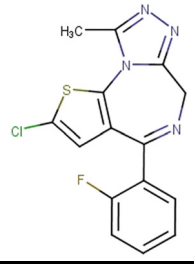
Chemical structure	Names
	**Clonazolam** or **Clonitrazolam** (6-(2-chlorophenyl)-1-methyl-8-nitro-4*H*-[1,2,4]triazolo[4,3-*a*][1,4] benzodiazepine
	**Flualprazolam** (8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4] diazepine
	**Flubromazolam** (8-bromo-6-(2-fluorophenyl)-1-methyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
	**Flunitrazolam** (6-(2-fluorophenyl)-1-methyl-8-nitro-4*H*-[1,2,4]triazolo[4,3-*a*][1,4] benzodiazepine
	**Nitrazolam** or **NitrazolaM** (1-methyl-8-nitro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4] benzodiazepine
	**Pyrazolam** (8-bromo-1-methyl-6-(pyridine-2-yl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4] benzodiazepine

Table 4: continued

Chemical structure	Names
	**Zapizolam** (8-chloro-6-(2-chlorophenyl)-4*H*-pyrido[2,3-*f*][1,2,4]triazolo [4,3-*a*][1,4] diazepine
	**Etizolam** (4-(2-chlorophenyl)-2-ethyl-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine
	**Deschloroetizolam** (4-phenyl-2-ethyl-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine
	**Metizolam** or **desmethyletizolam** (4-(2-chlorophenyl)-2-ethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*] [1,4]diazepine
	**Fluclotizolam** (2-chloro-4-(2-fluorophenyl)-9-methyl-6*H*-thieno[3,3-*f*][1,2,4]triazoleo[4,3-*a*] diazepine

*Phenazepam is used as a prescription medicine in Russian Federation, Estonia, Latvia, Lithuania and Belarus [456], and etizolam is used as a prescription medicine in Japan, India and Italy [457].

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Table 5: Metabolism of designer benzodiazepines [463]

Compound	Metabolites	Reference
Adinazolam	*In vitro* (HLM): *N*-desmethyladinazolam, *N*,*N*-didesmethyladinazolam	[464]
Diclazepam	*In vitro* (HLM): monohydroxylation → delorazepam, desmethylation → lormetazepam	[465]
	Human urine: delorazepam, lorazepam, lormetazepam	[465,466]
	Human serum: delorazepam	
Etizolam	*In vitro* (HLM): three monohydroxylated metabolites, keto-metabolite, etizolam glucuronide	[465,467]
	Post-mortem blood: α-hydroxyetizolam, 8-hydroxyetizolam	[468]
Deschloroetizolam	*In vitro* (HLM): hydroxydeschloroetizolam, dihydroxydeschloroetizolam, deschloroetizolam glucuronide	[465,467]
Flubromazolam	*In vitro* (HLM, human hepatocytes): 4-hydroxyflubromazolam, α-hydroxyflubromazolam, dihydroxyflubromazolam, flubromazolam glucuronide	[465,467,469,470]
	Human urine: α-hydroxyflubromazolam, 4-hydroxyflubromazolam, α-hydroxyflubromazolam glucuronide, α,4-dihydroxyflubromazolam, flubromazolam glucuronide	[469–472]
Metizolam	*In vitro* (HLM): 2-hydroxymetizolam, *N*-hydroxymetizolam, metizolam glucuronide	[464,467,473]
	Human urine: 2-hydroxymetizolam, *N*-hydroxymetizolam, 2-hydroxymetizolam glucuronide	[473]
Norflurazepam	*In vitro* (HLM): hydroxynorflurazepam, dihydroxynorflurazepam	[474]
Phenazepam	Human urine: 3-hydroxyphenazepam, 5-bromo-(2-chlorophenyl)-2-aminobenzophenone (ABPH), 6-bromo-(2-chlorophenyl) quinazoline-2-one (QNZ)	[456]
Pyrazolam	Human urine: pyrazolam glucuronide	[472]
Clonazolam	*In vitro* (HLM): aminoclonazolam, desmethylclonazolam, hydroxyclonazolam	[465]
	Human urine: 7-aminoclonazolam, 7-acetaminoclonazolam, hydroxyclonazolam, 7-aminoclonazolam glucuronide, 7-acetaminoclonazolam glucuronide, hydroxyclonazolam glucuronide	[475]
Cloniprazepam	*In vitro* (HLM): 7-aminocloniprazepam, hydroxycloniprazepam, dihydroxycloniprazepam, 3-ketocloniprazepam, clonazepam, 7-aminoclonazepam, hydroxyclonazepam, 3-hydroxy-7-aminoclonazepam, hydroxycloniprazepam glucuronide	[464,476]
Flunitrazolam	*In vitro* (HLM): hydroxyflunitrazolam, dihydroxyflunitrazolam, aminoflunitrazolam, flunitrazolam glucuronide	[467,474]
	Human urine: desnitroflunitrazolam, 7-aminoflunitrazolam, 7-acetamidoflunitrazolam, hydroxyflunitrazolam	[477]
Fonazepam (norflunitrazepam)	*In vitro* (HLM): 7-aminofonazepam (7-aminonorflunitrazepam), 3-hydroxyfonazepam (3-hydroxynorflunitrazepam; nifoxipam)	[464]
Meclonazepam	*In vitro* (HLM): aminomeclonazepam, hydroxymeclonazepam	[465]
	Human urine: 7-aminomeclonazepam, 7-acetaminomeclonazepam	[475,478]
Nifoxipam	*In vitro* (HLM): 7-aminonifoxipam, denitro-nifoxipam, nifoxipam glucuronide	[465,467]
Nitrazolam	*In vitro* (HLM): 8-aminonitrazolam, 4-hydroxynitrazolam/α-hydroxynitrazolam	[464]

AH-7921 (3,4-dichloro-*N*-[(1-dimethylamino)cyclohexylmethyl]benzamide) is a μ-opioid receptor agonist developed in 1974 by Allen and Hanburys Ltd. [448] and patented 2 years later as a potential analgesic agent [449]. The reported analgesic activity in mice is equal or slightly higher than that in morphine [450,451]. Studies on animals show that AH-7921 is approximately equipotent to morphine regarding antinociception, respiratory depression, sedation, Straub tail, decrease in pupil diameter, decrease in body temperature, and inhibition of gut propulsion [452].

AH-7921 has never been sold as a medicine due to its addictive properties [453], and it has no other industrial use.

Studies show that AH-7921 acts as an agonist at the κ and μ opioid receptors with a K_i of 50 and 10 nM, respectively [454].

3.7 Designer benzodiazepines

The DBZD include pharmaceutical drug candidates never been approved for medical use (deschloroetizolam, clonazolam, flubromazepam, diclazepam, pyrazolam, and meclonazepam) derivatives obtained by a simple modification of the registered drugs (flubromazolam), and some metabolites

Table 6: Structures of the global minima and their energies

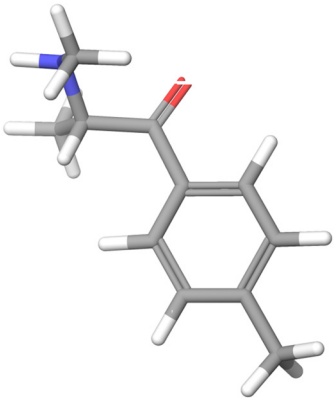
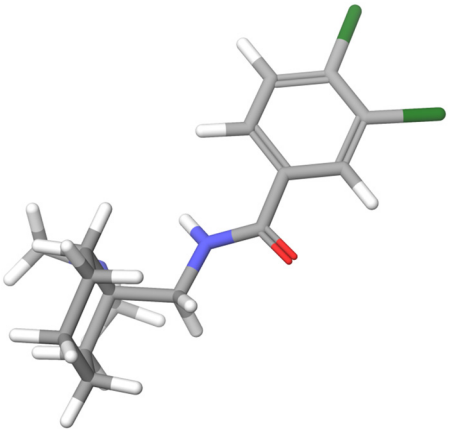
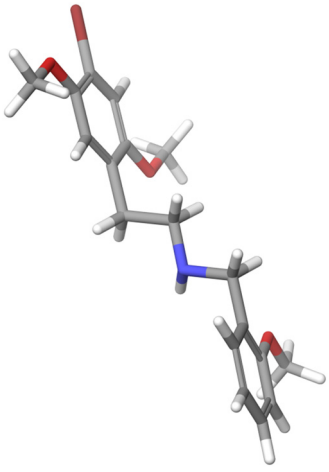
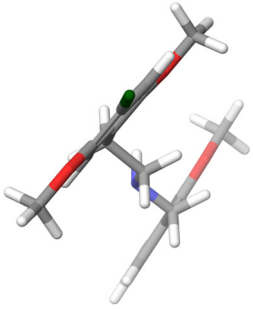
Name and structure of the global minimum of the compound	The energy of the global minimum, kJ/mol
Mephedrone 	276.15
AH-7921 	153.56
25B-NBOMe 	244.92
25C-NBOMe 	252.04

Table 6: continued

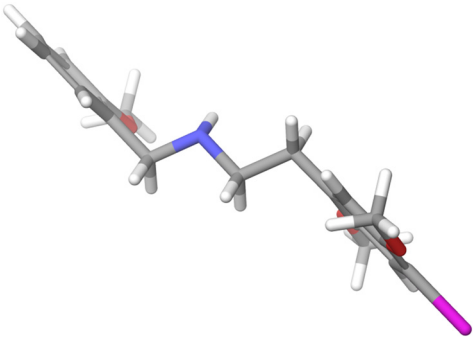
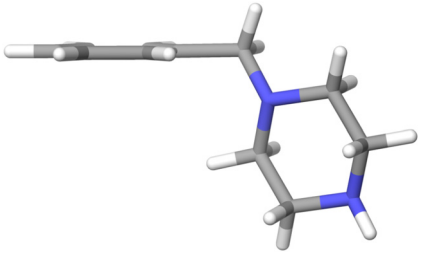
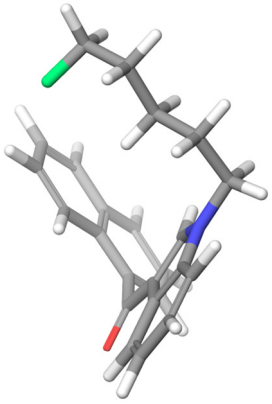
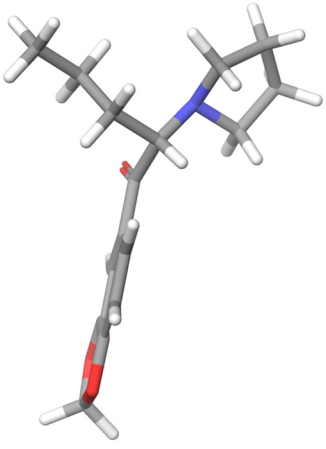
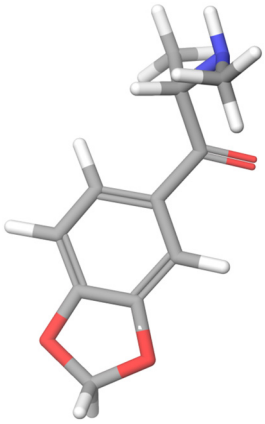
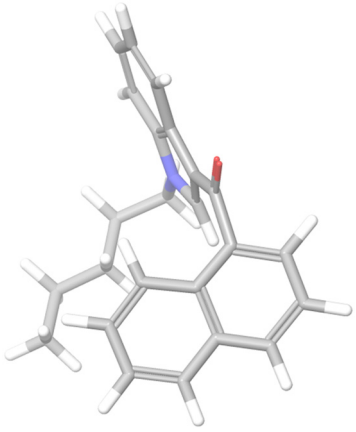
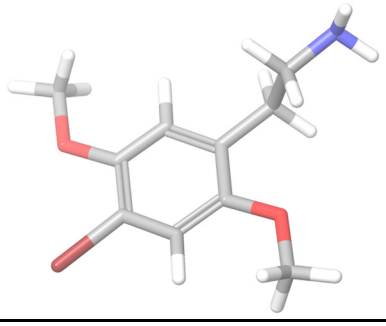
Name and structure of the global minimum of the compound	The energy of the global minimum, kJ/mol
25I-NBOMe 	244.16
BZP 	307.50
AM-2201 	345.15
MDPV 	265.43

Table 6: continued

Name and structure of the global minimum of the compound	The energy of the global minimum, kJ/mol
Methylone 	243.09
JWH-018 	340.96
2C-B 	143.71

of registered benzodiazepines (desmethylflunitrazepam and 3-hydroxydesmethylflunitrazepam) [455] (Table 4).

They appeared in early 1960s, and it was found that they act as positive allosteric modulators of GABA-A receptors, with the binding site at the α/γ subunit interface [455]. In medicine, DBZD are widely applied in the therapy of neurological and psychiatric disorders (e.g., panic attacks, anxiety, muscle spasms, insomnia, epilepsy, and alcohol withdrawal), and as a premedication prior to surgery and intraoperative medications [455].

The first recreationally used benzodiazepine was phenazepam in 2007, which was followed by etizolam in 2011 [457]. Phenazepam was created in the Soviet Union in the 1970s for the treatment of anxiety and alcohol withdrawal [456]. Etizolam was made initially in Japan (Depas) in 1984 as an anxiolytic medicine [455].

They are sold as tablets, capsules, pills, pellets, blotters, powders, and liquids [458–461].

Biological effects caused by benzodiazepines include increased muscle relaxation, sociability, feelings of well-being, and euphoria [455]. The common adverse effects of DBZD include impaired balance, somnolence, ataxia, impaired thinking and self-assessment capability, loss of coordination, slurred speech, muscle weakness, confusion, amnesia, dizziness, blurred vision, drowsiness, fatigue, lethargy, and palpitations [455]. High doses can induce auditory and visual hallucinations, delirium, deep sleep, seizures, and coma [455]. Long-acting flubromazolam users have sleeping paralysis, unpleasant night dreams, and somnambulistic states persisted for several days [462].

Three main ways are used to investigate the metabolism of DBZD like in the case of other NPS: (1) incubation of DBZD with human liver microsomes followed by the analysis of the metabolites, (2) analysis of urine samples of a large number of NPS users, and (3) analysis of urine samples in controlled self-administration studies [455]. The major biotransformation pathway for DBZD is in general oxidation and glucuronidation (Table 5) [463].

A solid-phase extraction and liquid-liquid extraction are used for sample clean-up and the extraction of DBZD. Certain DBZD have been found in blood and urine using immunochemical assays with high cross-reactivity (e.g., cloned enzyme donor immunoassay, enzyme multiplied immunoassay technique, enzyme-linked immunosorbent assay, and kinetic interaction of microparticles in solution) [467,479,480]. Immunochemical screening of biological specimens for DBZD has three major drawbacks: (1) NPS, especially the newest may be not detected when screened by immunoassay if they are not in the scope of the confirmation panel of benzodiazepines, (2) blood/serum levels of DBZD can be extremely low to be detected by immunoassays, and (3) cross-contamination [463,480].

Recently, an US-LDS-DLLME in combination with gas chromatography-triple quadruple mass spectrometry (GC-QQQ-MS) [481] and a nonaqueous capillary electrophoresis-tandem mass spectrometry [482] have been used for the detection of DBZD in urine and serum, respectively.

4 Statistical data on the use of illegal drugs

It seems that policies (especially reducing the open trade) on NPS have had an impact on the decrease in its number of the first detections in European countries. Currently, around 50 new substances are reported each year (55 in 2018), with over 730 reported to the EU Early Warning System [332]. Among the 731 registered today from 1997, there are 190 synthetic cannabinoids, 138 cathinones, 99 phenethylamines, 49 opioids, 42 tryptamines, 36 arylalkylamines, 28 benzodiazepines, 18 arylcyclohexylamines, 17 piperazines, 14 piperidines and pyrrolidines, 8 plants and extracts, 5 aminoindanes, and 87 other substances [332]. The number of users of NPS among young adults (15–34) goes from 0.1% in Norway to 3.2% in the Netherlands [332].

There are published papers on the detection of NPS in wastewater [483] or as a part of the doping control [38].

5 Conformational analysis

For the first time we show the spatial occupation and arrangements of the groups of illicit drugs. The investigated drugs (mephedrone, AH-7921, 25B-NBOMe, 25C-NBOMe, 25I-NBOMe, BZP, AM-2201, MDPV, methylone, JWH-018, and 2C-B) are relatively simple and small molecules, so conformational analysis is a reliable tool for the prediction of biologically active conformations (Table 6). The conformational analysis starting from the drawn structures of the illegal drugs (mephedrone, AH-7921, 25B-NBOMe, 25C-NBOMe, 25I-NBOMe, BZP, AM-2201, MDPV, methylone, JWH-018, and 2C-B) was performed in Macromodel, Schrodinger Suite 2016-1 [484] using MMFFs force field in water and chloroform. Initially drawn structures were first minimized in 10,000 steps, and then put for the conformational search. Nonbonded van der Waals cut-off was 8.0 Å, and all structures within 5 kcal/mol far from global minimum were saved. All other parameters were adjusted according to our previous investigations [485]. Images in Table 6 represent the most probable look of the investigated molecules in 3D. Also, the information about the energies of the global minima is presented. Obtained structures can be the initial steps in the further investigations of the interactions of illicit drugs with various biological targets.

We used before conformational analysis to predict physicochemical properties of selected illegal drugs [486].

Computational modeling was also performed to explain the interactions established between NPS (substituted cathinones and benzofurans) and transporters indicating the main amino acids in the binding pockets of transporters that effect drug affinities [487]. Similarly, molecular docking was used to predict interactions of selected synthetic cathinones with a complex of SAP97 PDZ2 with 5-HT2A receptor peptide [488]. Also, three

QSAR models were developed for the prediction of affinity of μ-opioid receptor ligands [489]. DFT calculations were shown as an efficient tool for the prediction of infrared and Raman spectra of newly synthesized cathinones [490].

6 Conclusion

This review article is an attempt to summarize the current state on the major used illicit drugs: their types, synthesis, metabolism, and identification. Currently, the number of reported NPS is decreasing each year due to the new EU policies with 50 new compounds in average. Investigations of NPS are increasing, so now we have a large and deep pool of data worldwide, both on natural and on synthetic NPS. Many of NPS were initially released from the official labs as medications against various diseases or were known for their use for religious or medicinal purposes. Searching for more efficient and less harmful antidotes should be a priority now. To the best of our knowledge, we are the first who performed the conformational analysis of selected NPS giving rise to the search of the biologically active conformations both theoretically and using lab experiments.

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