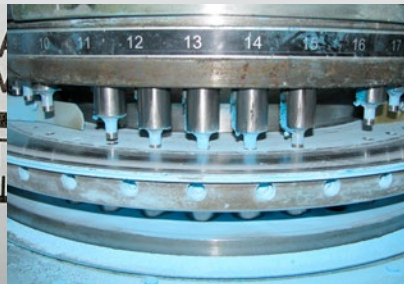
European Monitoring Centre
for Drugs and Drug Addiction

New benzodiazepines in Europe – a review



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Statement on the United Kingdom

The United Kingdom left the European Union on 1 February 2020. For the purpose of this report, the United Kingdom is not included in the term 'Member States'.

Methods and information sources

In the context of this report, 'new benzodiazepines' are defined as new psychoactive substances (NPS) that contain a benzodiazepine core, including structurally closely related compounds (e.g. thienodiazepines), and that are not controlled under the international drug control system. They also include three benzodiazepines – phenazepam, etizolam and flualprazolam – that were formerly classed as NPS but have recently been controlled under the international drug control system. Other common names for this category are 'designer benzodiazepines', 'NPS benzodiazepines' and, less frequently, 'synthetic benzodiazepine analogues'.

English-language articles were selected from a search of the PubMed and Web of Science databases performed on 18 June 2018 using the following search strings: (1) '(NPS OR "new psychoactive substance*") AND benzodiazepine*' and (2) 'designer benzodiazepine*'. Additional articles were identified from a review of the references cited in retrieved publications (snowball technique). Searches of selected medical specialty society and international, national and local government agency websites were conducted to identify clinical guidelines, position statements and reports. Search strings were introduced into Google and Google Scholar and the first 100 hits were screened to find additional relevant content. Although the systematic searches were conducted in 2018, information from the scientific papers and reports published in 2019 and 2020 was also included in this report. In particular, the most recent data on flualprazolam and etizolam were added. All the references in the European Database on New Drugs for substances listed in the benzodiazepine category were also included, except patents.

MEDLINE, PubMed, Google and internet platforms (e.g. Erowid, Bluelight, Eve and Rave) were searched for the terms 'designer benzodiazepines', 'NPS benzodiazepines', 'legal benzodiazepines', 'flubromazolam', 'flualprazolam', 'fluclotizolam', 'diclazepam', 'clonazolam', 'clonitrazolam', 'norfludiazepam' and 'bromazolam', alone or in combination, in May 2019. In addition, colleagues within our scientific network were contacted to obtain further information.

Information from the European Union Early Warning System on NPS (EWS), operated by the European Monitoring Centre for Drugs and Drug Addiction, has been included as relevant. The EWS is composed of a multiagency and multidisciplinary network, which includes the EMCDDA, 29 national early warning systems (27 EU Member States, Turkey, and Norway), Europol and its law enforcement networks, the European Medicines Agency (EMA), the European Commission, and other partners. Information from the United Nations system (the United Nations Office on Drugs and Crime and the World Health Organization), as well as from non-EU countries such as Canada, the United Kingdom and the United States, has also been included as relevant.

Executive summary

Benzodiazepines are one of the most important groups of medicines that are specifically used for sedation and to aid sleep. They are the most widely prescribed group of medicines in the world and are used to treat a range of conditions, including anxiety, insomnia, epilepsy and alcohol withdrawal. Benzodiazepines were introduced into clinical medicine in the early 1960s. They rapidly replaced barbiturates as sedative-hypnotics because they were safer and less likely to cause fatal central nervous system depression. Benzodiazepines act as central nervous system depressants by enhancing the actions of the neurotransmitter gamma-aminobutyric acid (GABA) on the benzodiazepine site of the GABA type A ($GABA_A$) receptor. Their effects include anxiolytic and sedative effects, muscle relaxation and anticonvulsive activity.

Since the mid 2000s, new benzodiazepines, which are not controlled under the international drug control system, have been sold as 'legal' replacements for controlled benzodiazepines in Europe. A small number of these new benzodiazepines, such as phenazepam and etizolam, are authorised medicines in some countries; many others may be found in the scientific and patent literature, but have never been authorised as medicines, and some are novel compounds. In Europe, new benzodiazepines are monitored as new psychoactive substances (NPS) by the European Union Early Warning System. This system is operated by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) as part of a three-step legal framework allowing the EU to rapidly detect, assess, and respond to public health and social threats caused by such substances.

Over the past few years, there has been an increase in the number and availability of new benzodiazepines on the drug market in Europe and, increasingly, in Canada and the United States. As of 28 February 2021, the EMCDDA was monitoring 30 new benzodiazepines through the EU Early Warning System. Of these, more than 80 % were detected for the first time between 2014 and 2020. Despite this relatively large number, the new benzodiazepine market in Europe is currently dominated by a handful of substances, most notably etizolam and flualprazolam, although this may change, as both substances were placed under international control in November 2020. In 2019, 1 240 seizures of new benzodiazepines were reported to the EU Early Warning System by the Member States, reflecting around 5 % of the total number of seizures of NPS.

Overall, these developments in the market give rise to concerns about both individual and public health for a number of reasons. The pharmacology and toxicology of new benzodiazepines is largely unknown, and the risks associated with their use may be higher than those associated with the use of authorised benzodiazepine medicines. In addition, the very nature of unregulated markets means that these risks may be amplified by the uncertain doses that are used. In some cases, users may not be aware that they are using these substances, and therefore might be at higher risk of severe poisoning, particularly if taken in combination with other central nervous system depressants, such as alcohol and opioids. Of particular concern is the growing use of new benzodiazepines to make falsified (fake) tablets of commonly prescribed benzodiazepine medicines, such as diazepam (Valium) and alprazolam (Xanax), and the involvement of criminal groups in producing such tablets. In some cases, the fake tablets are packaged in blister packs resembling legitimate products, which makes it more difficult for consumers to spot the fakes. Serious adverse events, such as severe poisonings, involving such fake medicines have been reported in Europe. Other risks might be related to the potential presence of adulterants, other substances or synthesis by-products from illicit manufacture and processing.

The reason for the increase in availability of new benzodiazepines in Europe is not entirely clear. In part, the increase mirrors the general increased availability of a range of NPS since the mid 2000s. In addition, given the widespread use of prescription benzodiazepines in society, and their diversion to the illicit drug market, the increase in new benzodiazepines might also be partially related to well-intentioned restrictions in the legal supply of authorised benzodiazepine medicines and the introduction of prescription limits to prevent or reduce harms among patients, such as dependence. While this is speculative, some support for this may come from the increasing number of fake benzodiazepine medicines that have been seized on the illicit drug market in the past few years that contain new benzodiazepines.

In the future, it is likely that new benzodiazepines with high potency and that are easy to synthesise will continue to be introduced into the market. In addition, there might be efforts to circumvent the (chemical) definition of generic approaches, as has been seen for other NPS.

The ongoing coronavirus disease 2019 (COVID-19) pandemic and related response measures may affect the existing benzodiazepine drug markets in unpredictable ways. Such effects may extend to changes in use and patterns of use of benzodiazepines, including a possible increase in prescriptions in order to treat insomnia and anxiety related to the pandemic. It may also lead to temporary shortages due to supply chain issues.

Ultimately, such changes may lead to a greater demand for new benzodiazepines, such as individuals seeking out new benzodiazepines to self-medicate, or inadvertently using new benzodiazepines from the use of fake medicines.

This report provides a technical review of the current body of knowledge regarding new benzodiazepines that are monitored by the EMCDDA. The aims of this report are to strengthen situational awareness of new benzodiazepines in Europe, and to help stakeholders prepare for and respond to public health and social threats caused by such substances.

Background

History of the development of benzodiazepines

The first benzodiazepine used as a medicine, chlordiazepoxide ([1]), was the accidental result of a research programme to develop new tranquillisers by the pharmaceutical company F. Hoffmann-La Roche AG in the 1950s. In the course of the research and development process, the chemist in charge, Leon Sternbach, and his team realised that, instead of the benzheptoxdiazines they had intended to synthesise, they had made quinazoline 3-oxides (Sternbach et al., 1979). The structure was subsequently determined as a 1,4-benzodiazepine.

Several 2-amino-1,4-benzodiazpine 4-oxides were patented in 1959, including chlordiazepoxide. The substance became commercially available as a medicine in 1960 under the trade name Librium, and it soon replaced the more toxic barbiturates to treat anxiety and sleep disorders. In 1963, diazepam (Valium) followed and it is still one of the most popular and widely prescribed benzodiazepines. Since then, a variety of structurally modified benzodiazepines have become available, with regional differences regarding the range of benzodiazepines approved by national medicine authorities.

Benzodiazepines are widely prescribed for various psychiatric disorders and have become indispensable medications in anaesthesiology and emergency care.

([1]) 7-Chloro-2-(methylamino)-5-phenyl-3*H*-1,4-benzodiazepine-4-oxide.

Legitimate uses of benzodiazepines

Benzodiazepines used as medicines are produced by licensed pharmaceutical companies and authorised and marketed according to national legislation. In most countries, benzodiazepines are prescription-only medicines and are subject to additional restrictions on their supply, use and possession under drug control laws.

Benzodiazepines are used to treat a range of conditions, including anxiety, insomnia, epilepsy and muscle spasms, and to manage withdrawal symptoms from alcohol. In addition, they are also used as premedication prior to or during surgical procedures (short-term sedation), and for analgosedation ([2]) in intensive care. The varying prescribing patterns of therapeutic application of benzodiazepines are caused by the fact that, despite qualitatively similar clinical effects, there are important quantitative differences in the pharmacodynamic spectra and pharmacokinetic properties of different benzodiazepines.

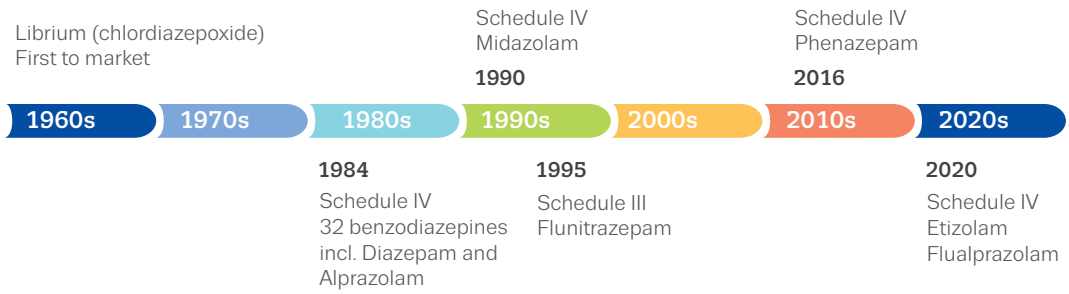
Benzodiazepines are recommended for short-term use at the lowest possible dose in order to reduce the risks of tolerance, dependence and withdrawal symptoms.

International control measures

In most countries, benzodiazepines authorised as medicines are controlled under drug control laws and are available by prescription only. This is in agreement with the 1971 United Nations Convention on Psychotropic Substances, which currently controls 38 benzodiazepines ([3]). These are alprazolam, bromazepam, brotizolam, camazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, clotiazepam, cloxazolam, delorazepam, diazepam, estazolam, ethyl loflazepate, etizolam (since 2020), flualprazolam (since 2020), fludiazepam, flurazepam, flunitrazepam, halazepam, haloxazolam, ketazolam, loprazolam, lorazepam, lormetazepam, medazepam, midazolam, nimetazepam, nitrazepam, nordazepam, oxazepam, oxazolam, phenazepam (since 2016), pinazepam, prazepam, temazepam, tetrazepam and triazolam (Figure 1).

([2]) Analgosedation is a sedative-minimising technique; although not eliminating the use of sedatives entirely, it prioritises pain control and analgesia use, saving sedative agents for rescue therapy only.
([3]) A total of 37 of them are under Schedule IV (flunitrazepam was transferred to Schedule III in 1995).

FIGURE 1
Timeline of the international control status of benzodiazepines



New benzodiazepines in Europe

In the context of this report, 'new benzodiazepines' are defined as NPS that contain a benzodiazepine core, including structurally closely related compounds (e.g. thienodiazepines), and that are not controlled under the international drug control system. They also include three benzodiazepines – phenazepam, etizolam and flualprazolam – that were formerly classed as NPS but have recently been controlled under the international drug control system.

The term 'designer benzodiazepine' has been used in analogy to 'designer drugs', based on the idea of intentionally modifying an established pharmaceutical or illicit drug to circumvent national and/or international control measures. Other terms used include 'novel benzodiazepines' (EMCDDA, 2017) or 'new research benzodiazepines' (Wohlfarth et al., 2017). Most new benzodiazepines were described in patent or scientific literature before their emergence in the drugs market.

Emergence of new benzodiazepines

In Europe, the EU Early Warning System, operated by the EMCDDA, monitors and responds to the appearance of NPS, under the terms of Regulation (EC) No 1920/2006 (as amended by Regulation (EU) 2017/2101) ([4]) (EMCDDA, 2019a) and Council Framework Decision 2004/757/JHA (as amended by Directive (EU) 2017/2103).

As of 28 February 2021, the EMCDDA was monitoring 30 new benzodiazepines through the EU Early Warning System (Figure 2) (EMCDDA, 2020). Despite this relatively large number, the market in Europe is dominated by a handful of substances. In the past few years, etizolam and flualprazolam, in particular, have played an increasingly important role in this market in some parts of Europe, especially in the manufacture of fake benzodiazepine medicines, such as diazepam and alprazolam tablets (EMCDDA, 2019b; EMCDDA, 2019c; Nielsen and McAuley, 2020). However, this may change, as etizolam and flualprazolam were placed under international control in November 2020.

New benzodiazepines notified to the EU Early Warning System

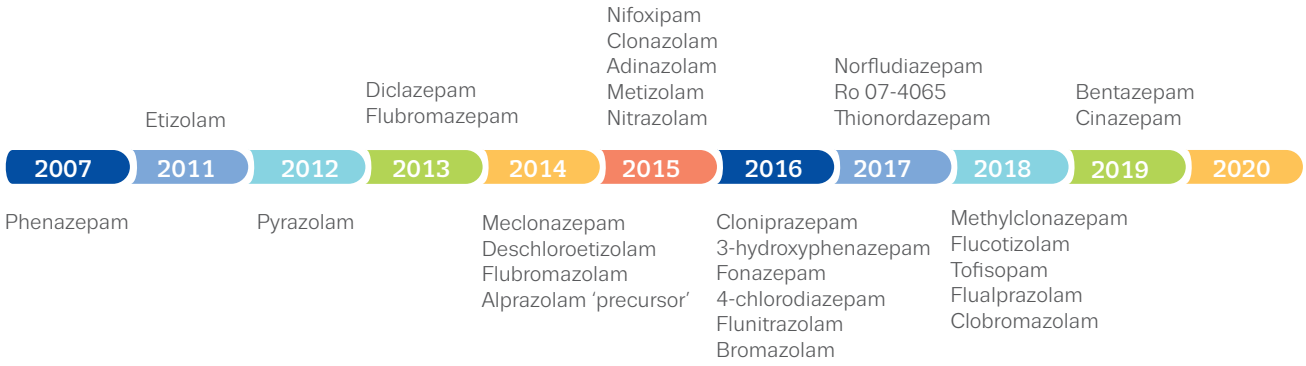
The first new benzodiazepine to appear on the drugs market in Europe was phenazepam in 2007 (EMCDDA, 2017) ([5]). It was typically sold as a 'legal high' but was also deliberately mis-sold as, or used to make, fake versions of benzodiazepine medicines in some countries. Phenazepam was originally developed as a medicine in Russia (then the USSR) in 1975, and continues to be marketed as such in Russia and other countries of the former Soviet Union. The identification of phenazepam on the European drugs market was followed by that of etizolam in 2011 (EMCDDA and Europol, 2012). Like phenazepam, etizolam is also authorised as a medicine in some countries. Between 2008 and 2011, both phenazepam and etizolam were subjected to control measures in several Scandinavian countries (e.g. Sweden, Norway and Finland). Following this, other new, unregulated, benzodiazepines started to be sold on the 'legal high' market in Europe.

Pyrazolam ([6]) was the first 'designer benzodiazepine' advertised for sale online from mid 2012 on (EMCDDA and Europol, 2013; Moosmann et al., 2013a). The compound resembles alprazolam, with the phenyl moiety substituted by a pyridinyl moiety and bromine, instead of chlorine

([4]) Regulation (EU) 2017/2101 of the European Parliament and of the Council of 15 November 2017 amending Regulation (EC) No 1920/2006 as regards information exchange on, and an early warning system and risk assessment procedure for, new psychoactive substances, OJ L 305, 21.11.2017, p. 1 (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32017R2101).

([5]) Феназепам.
([6]) 8-Bromo-1-methyl-6-(pyridin-2-yl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine.

FIGURE 2
Timeline of benzodiazepines formally notified to the EU Early Warning System for the first time, 2007–2020



attached to C_8. Flubromazepam ([7]) and diclazepam ([8]) followed pyrazolam onto the market in 2013. Following this, an increasing number of new benzodiazepines emerged on the market in Europe, bringing to 30 the total number monitored as of 28 February 2021 (Figure 2).

The 30 new benzodiazepines identified so far in Europe are listed in Table 1 on page 8, together with information on their chemical structure, year of appearance, main phase I metabolites and relevant references (Moosmann and Auwärter, 2018).

In 2019, the EMCDDA issued risk communications on flualprazolam (EMCDDA, 2019b) and etizolam (EMCDDA, 2019c) to the Early Warning System Network that highlighted concerns related to increased availability of the substances in Europe, reported harms, and their use to make fake tablets of commonly prescribed benzodiazepine medicines.

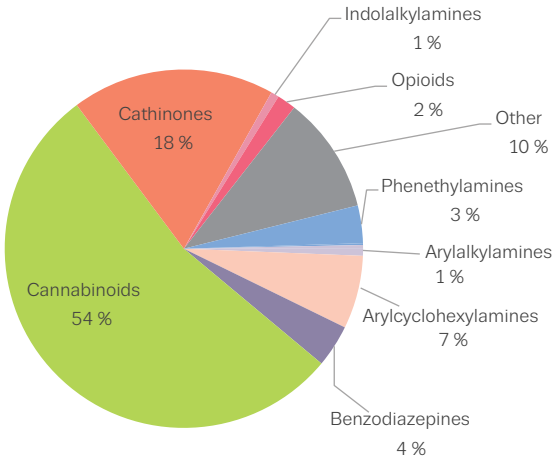
Other possible new benzodiazepines

Although it is unclear why some new benzodiazepines have come to dominate the market, and not others, it is noteworthy that two of them, phenazepam and etizolam, are authorised medicines. It is possible that other benzodiazepines authorised as medicines and not under international control might emerge as new benzodiazepines in the European drug market in the future. These include flutazolam, which is structurally related to the controlled haloxazolam and is used as a medicine in Japan; cinolazepam (available in, for example, Austria, Bulgaria, Czechia, Hungary, Slovakia and Romania); doxefazepam (e.g. available in Italy); flutoprazepam (e.g. available in Japan); gidazepam (available in Russia and Ukraine); metaclazepam (formerly available in Germany and Austria, but no longer marketed); mexazolam (available in, for example, Japan and Portugal); and quazepam (available in, for example, the United States, Japan and Spain).

Availability, size of the market

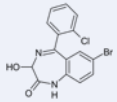
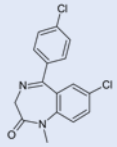
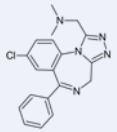
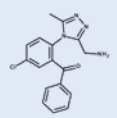
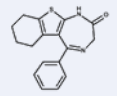
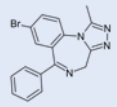
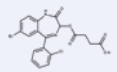
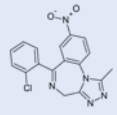
The size of the new benzodiazepines market is not known. As estimated from the seizures of police and customs authorities, new benzodiazepines do not seem to play a major role when compared with other NPS groups such as synthetic cannabinoids or cathinones (Figure 3). In 2019, 1 334 seizures of new benzodiazepines were reported to the EU Early Warning System (1 240 (93 %) of which were reported by the Member States), reflecting around 3.8 % of the total number of seizures of NPS (5.6 % in the Member States).

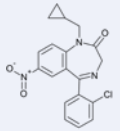
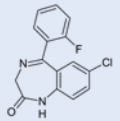
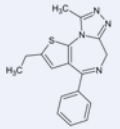
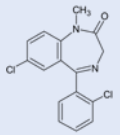
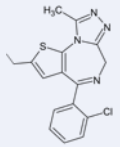
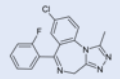
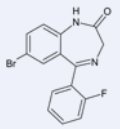
FIGURE 3
Seizures of NPS reported to the EU Early Warning System, 2005–2019: percentage per category (EU-27 + 2)

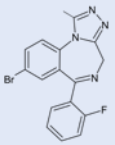
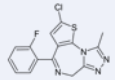
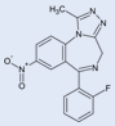
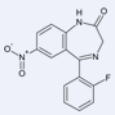
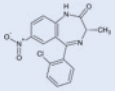
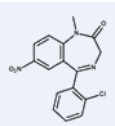
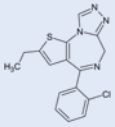
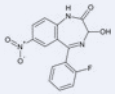
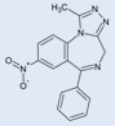


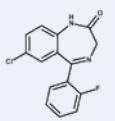
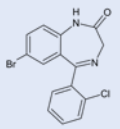
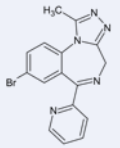
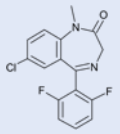
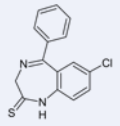
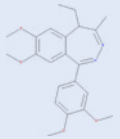
([7]) 7-Bromo-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one.
([8]) 7-Chloro-5-(2-chlorophenyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

TABLE 1
Summary of chemical and pharmacological information, including molecular structure, on new benzodiazepines

Substance	Systematic name (IUPAC)	Chemical classification	Formula	Molecular structure	Year identified	Major phase I metabolites (*in vivo*, *in vitro*)	References
3-Hydroxyphenazepam	7-Bromo-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one	1,4-Benzodiazepine	$C_{15}H_{10}BrClN_2O_2$		2015	N/A	Moosmann et al., 2016
4'-Chlorodiazepam (Ro5-4864)	7-Chloro-5-(4-chlorophenyl)-1-methyl-3*H*-1,4-benzodiazepin-2-one	1,4-Benzodiazepine	$C_{16}H_{12}Cl_2N_2O$		2016	Nor-Ro5-4864, hydroxy-Ro5-4864, hydroxy-nor-Ro5-4864	Moosmann et al., 2016
Adinazolam	1-(8-Chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,5-*a*] [1,4]benzodiazepin-1-yl)-*N*,*N*-dimethylmethanamine	Triazolobenzodiazepine	$C_{19}H_{18}ClN_5$		2015	*N*-desmethyladinazolam, *N*,*N*-didesmethyladinazolam, α-hydroxyalprazolam, estazolam	Lahti et al., 1983; Fraser et al., 1993; Moosmann et al., 2016
Alprazolam triazolobenzophenone derivative	[2-[3-(aminomethyl)-5-methyl-4H-1,2,4-triazol-4-yl]-5-chlorophenyl] phenyl-methanone	Triazolobenzodiazepine	$C_{17}H_{15}ClN_4O$		2014	Metabolite of alprazolam	Cho et al., 1986
Bentazepam	5-phenyl-1,3,6,7,8,9-hexahydro-2*H*-[1]benzothieno[2,3-e][1,4]diazepin-2-one	Thienodiazepine	$C_{17}H_{16}N_2OS$		2019	N/A	Gonzalez López et al., 1986
Bromazolam	8-Bromo-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine	Triazolobenzodiazepine	$C_{17}H_{13}BrN_4$		2016	N/A	
Cinazepam	4-[[7-bromo-5-(2-chlorophenyl)-2-oxo-1,3-dihydro-1,4-benzodiazepin-3-yl]oxy]-4-oxo-butanoic acid	1,4-Benzodiazepine	$C_{19}H_{14}BrClN_2O_5$		2019	3-Hydroxyphenazepam	Schukin et al., 2011
Clonazolam	6-(2-Chlorophenyl)-1-methyl-8-nitro-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine	Triazolobenzodiazepine	$C_{17}H_{12}ClN_5O_2$		2014	8-Aminoclonazolam, 8-acetamidoclonazolam, hydroxyclonazolam	Huppertz et al., 2015; Meyer et al., 2016; El Balkhi et al., 2017

Substance	Systematic name (IUPAC)	Chemical classification	Formula	Molecular structure	Year identified	Major phase I metabolites (*in vivo*, *in vitro*)	References
Cloniprazepam	5-(2-Chlorophenyl)-1-(cyclopropylmethyl)-7-nitro-1,3-dihydro-2*H*-[1,4]-benzodiazepin-2-one	1,4-Benzodiazepine	$C_{19}H_{16}ClN_3O_3$		2015	Hydroxycloniprazepam, dihydroxycloniprazepam, 7-aminocloniprazepam, ketocloniprazepam, clonazepam (dealkylcloniprazepam), 7-aminoclonazepam, 3-hydroxy-7-aminoclonazepam, two further hydroxyclonazepam metabolites (presumably at positions 3 and 4')	Moosmann et al., 2016; Mortelé et al., 2018
N-Desalkylflurazepam (norflurazepam)	7-Chloro-5-(2-fluorophenyl)-1,3-dihydro-1,4-benzodiazepin-2-one	1,4-Benzodiazepine	$C_{15}H_{10}ClFN_2O$		2016	Two hydroxynorflurazepam metabolites (presumably at positions 3 and 4'), dihydroxynorflurazepam; possibly no phase I metabolites at all (only phase II, similar to flurazepam)	Breimer and Jochemsen, 1983; unpublished data (Moosmann, Auwärter)
Deschloroetizolam	2-Ethyl-9-methyl-4-phenyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine	Thienotriazolodiazepine	$C_{17}H_{16}N_4S$		2014	Hydroxydeschloroetizolam (suggested 9-methyl position), hydroxydeschloroetizolam (suggested 2-ethyl position), hydroxydeschloroetizolam (suggested C-6 position), dihydroxydeschloroetizolam (positions unknown)	Huppertz et al., 2015; El Balkhi et al., 2017
Diclazepam (Ro5-3448)	7-Chloro-5-(2-chlorophenyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one	1,4-Benzodiazepine	$C_{16}H_{12}Cl_2N_2O$		2013	Lormetazepam, delorazepam, lorazepam	Moosmann et al., 2014;El Balkhi et al., 2017
Etizolam	4-(2-Chlorophenyl)-2-ethyl-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine	Thienotriazolodiazepine	$C_{17}H_{15}ClN_4S$		2011	α-Hydroxyetizolam, 8-hydroxyetizolam	El Balkhi et al., 2017
Flualprazolam	8-Chloro-6-(2-fluorophenyl)-1-methyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine	Triazolobenzodiazepine	$C_{17}H_{12}ClFN_4$		2017	N/A	N/A
Flubromazepam	7-Bromo-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one	1,4-Benzodiazepine	$C_{15}H_{10}BrFN_2O$		2013	3-Hydroxyflubromazepam, debromoflubromazepam, hydroxyflubromazepam	Moosmann et al., 2013b; El Balkhi et al., 2017

Substance	Systematic name (IUPAC)	Chemical classification	Formula	Molecular structure	Year identified	Major phase I metabolites (*in vivo*, *in vitro*)	References
Flubromazolam	8-Bromo-6-(2-fluorophenyl)-1-methyl-4*H*-[1,2,4]triazolo-[4,3-*a*][1,4] benzodiazepine	Triazoloben zodiazepine	$C_{17}H_{12}BrFN_4$		2014	α-Hydroxyflubromazolam, 4-hydroxyflubromazolam, hydroxyflubromazolam (position unknown), α,4-dihydroxyflubromazolam	El Balkhi et al.,2017; Noble et al., 2017; Pettersson Bergstrand et al., 2018; Wohlfarth et al., 2017; Huppertz et al., 2015; Huppertz et al., 2018
Fluclotizolam	2-Chloro-4-(2-fluorophenyl)-9-methyl-6*H*-thieno[3,2-*f*][1,2,4] triazolo[4,3-*a*][1,4]diazepine	Thienotriazolo-diazepine	$C_{15}H_{10}ClFN_4S$		2017	N/A	
Flunitrazolam	6-(2-Fluorophenyl)-1-methyl-8-nitro-4*H*-[1,2,4]triazolo[4,3-*a*][1,4] benzodiazepine	Triazoloben zodiazepine	$C_{17}H_{12}FN_5O_2$		2016	8-Aminoflunitrazolam, hydroxyflunitrazolam (suggested α-position), hydroxyflunitrazolam (suggested 4-position), dihydroxyflunitrazolam (suggested α-position and 4-position), 8-aminohydroxyflunitrazolam (suggested α-position or 4-position)	Unpublished data (Moosmann, Auwärter)
Fonazepam (desmethyl-flunitrazepam)	5-(2-Fluorophenyl)-1,3-dihydro-7-nitro-2*H*-1,4-benzodiazepin-2-one	1,4-Benzo-diazepine	$C_{15}H_{10}FN_3O_3$		2016	7-Aminofonazepam, 3-hydroxyfonazepam (nifoxipam), hydroxyfonazepam (suggested 4-position)	Moosmann et al., 2016
Meclonazepam	(3*S*)-5-(2-Chlorophenyl)-3-methyl-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one	1,4-Benzo-diazepine	$C_{16}H_{12}ClN_3O_3$		2014	7-Aminomeclonazepam, 7-acetaminomeclonazepam, hydroxymeclonazepam	Huppertz et al., 2015; Meyer et al., 2016; El Balkhi et al., 2017; Vikingsson et al., 2017
Methyl clonazepam	5-(2-chlorophenyl)-1-methyl-7-nitro-3*H*-1,4-benzodiazepin-2-one	1,4-Benzo-diazepine	$C_{16}H_{12}ClN_3O_3$		2018	N/A	
Metizolam (desmethyl-etizolam)	4-(2-Chlorophenyl)-2-ethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*] [1,4]diazepine	Thienotriazolo-diazepine	$C_{16}H_{13}ClN_4S$		2015	Two hydroxymetizolam metabolites (position undetermined; most likely 2-ethyl or 6-position); dihydroxymetizolam (positions unknown)	Moosmann et al., 2016; Kintz et al., 2017
Nifoxipam	5-(2-Fluorophenyl)-3-hydroxy-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one	1,4-Benzo-diazepine	$C_{15}H_{10}FN_3O_4$		2014	7-Aminonifoxipam, 7-acetamidonifoxipam	Meyer et al., 2016; El Balkhi et al., 2017
Nitrazolam	1-Methyl-8-nitro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4] benzodiazepine	Triazolobenzo-diazepine	$C_{17}H_{13}N_5O_2$		2015	Hydroxynitrazolam (suggested α-position or 4-position), 8-aminonitrazolam	Moosmann et al., 2016

Substance	Systematic name (IUPAC)	Chemical classification	Formula	Molecular structure	Year identified	Major phase I metabolites (*in vivo*, *in vitro*)	References
Norfludiazepam	7-chloro-5-(2-fluorophenyl)-1,3-dihydro-1,4-benzodiazepin-2-one	1,4-Benzodiazepine	$C_{15}H_{10}ClFN_2O$		2017	Metabolite of flurazepam	Vogt et al., 2013
Phenazepam	7-Bromo-5-(2-chlorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one	1,4-Benzodiazepine	$C_{15}H_{10}BrClN_2O$		2007	3-Hydroxyphenazepam, hydroxy-methoxy metabolite (positions unknown)	Zherdev et al., 1982; Maskell et al., 2012
Pyrazolam	8-Bromo-1-methyl-6-(pyridin-2-yl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine	Triazolobenzodiazepine	$C_{15}H_{10}BrN_5$		2012	α-Hydroxypyrazolam, 4-hydroxypyrazolam	Moosmann et al., 2013b; Pettersson Bergstrand et al., 2018
Ro7-4065 (difludiazepam)	7-Chloro-5-(2,6-difluorophenyl)-1-methyl-3*H*-1,4-benzodiazepin-2-one	1,4-Benzodiazepine	$C_{16}H_{11}ClF_2N_2O$		2017	N/A	
Thionordazepam	7-Chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-thione	1,4-Benzodiazepine	$C_{15}H_{11}ClN_2S$		2017	N/A	
Tofisopam	1-(3,4-dimethoxyphenyl)-5-ethyl-7,8-dimethoxy-4-methyl-5*H*-2,3-benzodiazepine	2,3-benzodiazepine	$C_{22}H_{26}N_2O_4$		2018	N/A	

NB: IUPAC, International Union of Pure and Applied Chemistry; N/A, no data available.

International legal response to new benzodiazepines

As of February 2021, none of the new benzodiazepines monitored by the EMCDDA had been risk assessed at the EU level.

In 2015, the Expert Committee on Drug Dependence (ECDD) of the World Health Organization (WHO) pre-reviewed phenazepam. Following analysis of the information provided in the pre-review report, it was decided that there was sufficient evidence of dependence and harm to proceed directly to a critical review during the meeting (WHO, 2016). In 2016, the United Nations Commission on Narcotic Drugs (CND) followed the recommendation of the ECDD to add phenazepam to the list of controlled substances under Schedule IV of the 1971 United Nations Convention on Psychotropic Substances.

The ECDD reviewed etizolam at its 26th meeting in 1989 (WHO, 1989) and at its 27th meeting in 1990 (WHO, 1991). At the 37th ECDD meeting in 2015, the committee pre-reviewed etizolam and recommended that a critical review was warranted (WHO, 2016). Etizolam was critically reviewed at the 39th ECDD meeting in 2017 and was recommended to remain under surveillance considering the insufficiency of data regarding dependence, abuse and risks to public health (WHO, 2018).

In 2019, etizolam (WHO, 2020a) and flualprazolam (WHO, 2020a) were assessed at the 42nd meeting of the ECDD. Subsequently, both etizolam and flualprazolam were placed under international control at the 63rd session of the CND in 2020, following the recommendation of the ECDD to add them to Schedule IV of the 1971 United Nations Convention on Psychotropic Substances (UNODC, 2020; WHO, 2020a). These control measures came into effect on 3 November 2020 (UNODC, 2020).

In 2020, clonazolam (WHO, 2020b), diclazepam (WHO, 2020c) and flubromazolam (WHO, 2020d) were assessed at the 43rd meeting of the ECDD and were recommended to be included in Schedule IV of the 1971 United Nations Convention on Psychotropic Substances.

Case study of a national response: Germany

In Germany, many prescription benzodiazepines are controlled by the 'Betäubungsmittelgesetz' (BtMG) (Narcotics Law) and listed in Annex III to the BtMG (marketable or prescribed drugs). Pharmaceutical drug preparations containing benzodiazepines up to certain maximum levels are usually exempted from the narcotic prescription regulation ('exempted preparations') and can be prescribed on a regular prescription. Specified maximum quantities per pharmaceutical form (tablet, suppository, ampoule, volume unit for drops) and/or maximum quantities per package are also regulated. Maximum amounts are defined individually for each active substance (e.g. for alprazolam, it is up to 1 mg), and result from Annex III to the BtMG. Quantities exceeding the stated amounts require a narcotic drug prescription, which is associated with increased bureaucratic expenditure and is monitored by the corresponding national authority, the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte). Substances controlled by the BtMG without such exemptions are camazepam, cloxazolam, delorazepam, ethyl loflazepate, etizolam, fludiazepam, flunitrazepam, haloxazolam, nimetazepam, phenazepam and pinazepam (etizolam and phenazepam were added in July 2013). New benzodiazepines such as diclazepam and flubromazepam were added to Annex II to the BtMG in November 2015.

Benzodiazepines that have recently appeared on the German market were not covered by this legislation. In response, the annex to the German law on NPS (Neue-psychoaktive-Stoffe-Gesetz (NpSG)) was extended to include several groups of substances, among them the group of benzodiazepines generically defined by their chemical structure, in July 2019. This includes 1,4-benzodiazepines, 1,5-benzodiazepines, their triazolo and imidazolo derivatives and a number of structural subgroups (loprazolam-, ketazolam-, oxazolam- and chlordiazepoxide-line compounds). In contrast to the BtMG, the NpSG allows criminal prosecution only for trafficking drugs; the purchase and possession of drugs for self-use are not covered under penal law, although seizures of these substances by law enforcement are authorised.

Replacement

It is possible that other new benzodiazepines will replace recently controlled benzodiazepines, such as etizolam and flualprazolam, on the NPS market. One possibility is the re-emergence of flubromazolam and deschloroetizolam, which were first notified in Europe in 2014 (EMCDDA and Europol, 2015). Data from the Welsh Emerging Drugs and Identification of Novel Substances Project (Wedinos) appear to suggest that flubromazolam and deschloroetizolam have recently re-emerged on the drug market in the United Kingdom and are being used to make fake diazepam, fake alprazolam, fake temazepam and fake zopiclone (Wedinos, 2020). In the United States, there has also been a recent increase in detections of these substances in forensic cases, including drug-related deaths (Center for Forensic Science Research and Education, 2021).

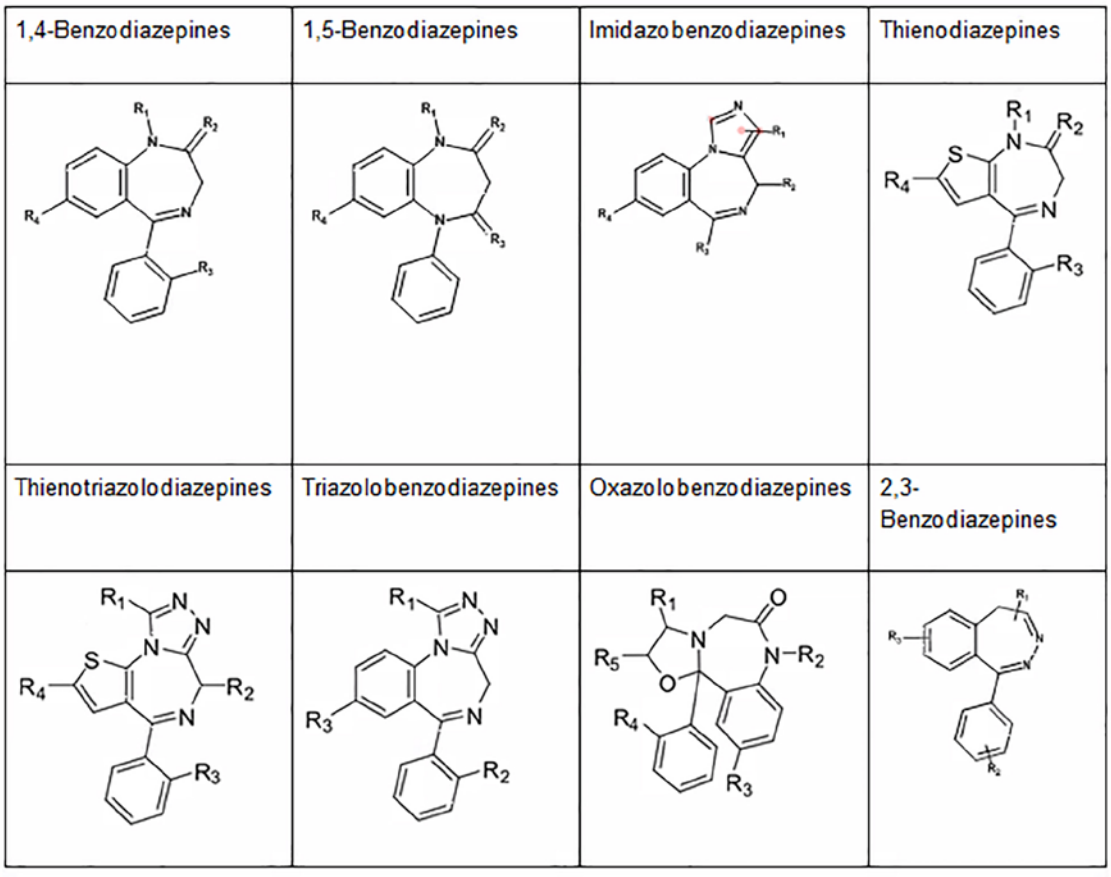
Physical, chemical and pharmacological properties

Physical and chemical description

All compounds classified as 'benzodiazepines' have a shared core structure and, according to their chemical structure and substitution pattern, fall into one of several subgroups (Figure 4):

- 1,4-benzodiazepines
- 1,5-benzodiazepines
- imidazobenzodiazepines
- thienodiazepines
- thienotriazolodiazepines
- triazolobenzodiazepines
- oxazolobenzodiazepines
- 2,3-benzodiazepines.

FIGURE 4
The structural cores of several subgroups of benzodiazepines and indicative Markush structures



At the time of publishing this report, all new benzodiazepines that have appeared on the drug market belong to five of the aforementioned groups: 1,4-benzodiazepines, 2,3-benzodiazepines, thienodiazepines, thienotriazolodiazepines and triazolobenzodiazepines.

Physical description

Most of the new benzodiazepines are described as white and odourless crystalline powders in their pure form. Less pure substances may show yellowish discolouration and an unpleasant 'chemical' smell due to residual solvents and impurities from synthesis. Benzodiazepines are rather lipophilic drugs, with very limited solubility in water and non-polar aliphatic solvents. Their solubility in aliphatic alcohols or more polar solvents, such as chloroform, is much greater. For the new benzodizepines, typical octanol–water partition coefficients (expressed as log K_{OW} or log P) at pH 7.4 (log $D_{7.4}$) are between 1 (e.g. pyrazolam) and 3.25 (e.g. phenazepam).

Chemical stability

In general, pure and dried benzodiazepines can be regarded as chemically stable. However, in solutions, they can be unstable, particularly if they contain structural elements such as a nitro moiety. The stability of benzodiazepines in different solvents and biological matrices, stored at temperatures of between 20 °C and –80 °C, has been addressed in several studies (Melo et al., 2012; Skopp et al., 1998; Skopp, 2004). The most unstable compounds were shown to be representatives of the nitrobenzodiazepine family (Robertson and Drummer, 1998). Pettersson Bergstrand et al. (2016) investigated the stability in urine for 1 month of the following new benzodiazepines: clonazolam, deschloroetizolam, diclazepam, etizolam, flubromazepam, flubromazolam, flutazolam, meclonazepam, nifoxipam, phenazepam and pyrazolam. Flubromazepam and the nitrobenzodiazepines clonazolam, meclonazepam and nifoxipam were unstable in urine at ambient temperature and at – 4 °C. After 4 weeks at – 4 °C, the meclonazepam concentration decreased to 8 % of its initial value. Another study showed that meclonazepam and 3-methylclonazepam were unstable in plasma when stored in glass, but not when stored in polypropylene tubes at –20 °C (Coassolo et al., 1985). Although the data published so far suggest a possible instability of any newly emerging nitrobenzodiazepines, it is recommended to investigate new compounds for their analytical stability. The ideal sample storage should be –20 °C or lower prior to analysis and collection tubes should be chosen with care (e.g. polypropylene versus glass; addition of sodium fluoride or fluoride oxalate).

Methods for identification and analysis

Owing to the extended conjugated π-electron system, the chemical identification of benzodiazepines can generally be carried out by ultraviolet (UV) spectroscopy, although the UV spectra can be very similar for compounds with the same chromophore, thereby requiring a chromatographic separation for unambiguous identification. Mass spectrometric techniques, nuclear magnetic resonance spectroscopy and infrared or Raman spectroscopy are also suitable for the chemical identification of benzodiazepines. For the analysis of biological samples, gas chromatography–tandem mass spectrometry (GC-MS/MS) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) are usually applied. Immunoassay screening can also be applied successfully (Pettersson Bergstrand et al., 2017), unless the expected concentrations are very low, as is the case for extremely potent newer benzodiazepines such as flunitrazolam (Ameline et al., 2019). However, in the case of a positive immunochemical assay, laboratories have to be prepared to detect the whole range of new benzodiazepines for confirmation. Otherwise, the finding might be incorrectly interpreted as a 'false positive'.

Manufacturing methods and chemical precursors

The synthesis of new benzodiazepines can include the introduction of a triazolo ring to precursor 1,4-benzodiazepines (Kleemann et al., 2009), which are readily available as pure substances because of their pharmaceutical use. Examples are pyrazolam (from bromazepam), clonazolam (from clonazepam) and nitrazolam (from nitrazepam). Owing to the generally greater potency of the corresponding triazolo derivatives, modifications of new benzodiazepines already sold on the NPS market have been achieved in this way, for example flubromazolam (from flubromazepam), flualprazolam (from norfludiazepam, also known as norflurazepam) and flunitrazolam (from fonazepam, also known as norflunitrazepam). While various other routes have been described, a typical synthesis route of 1,4-benzodiazepines involves the use of halogenated 2-amino-benzophenone, which is reacted with methyl 2-aminoacetate under cyclisation.

Physical and pharmaceutical form

Prescription benzodiazepines are most commonly available as tablets, capsules, solutions for injection/infusion, oral solutions and suppositories.

New benzodiazepines are marketed as 'research chemicals' in the form of often highly pure, crystalline powders, but also as tablets, capsules or in blotter form. They are sometimes available in different strengths that seem to be adjusted to the potency of the substance, as is the case with medicinal products containing benzodiazepines. A few studies have examined the strength of products containing new benzodiazepines sold on the NPS market. These show that, in at least some cases, the products contained the declared amount of the substance (Moosmann et al., 2013a; Moosmann et al., 2013b); however, large variances were reported in other cases, for example 0.59–1.39 mg (mean 0.94 mg; *n* = 13) for diclazepam tablets declared to contain 1 mg (Moosmann et al., 2014). In addition, new benzodiazepines are increasingly used to make fake versions of commonly prescribed medicines such as alprazolam and diazepam tablets. The strength of these fake products is rarely reported.

Pharmacology

The GABA system and benzodiazepine receptors

Benzodiazepines mainly act via the GABA receptor family, targeting the central $GABA_A$ receptor located in post- and presynaptic membranes (Mohsin and Qadir, 2015).

$GABA_A$ receptors are ligand-gated ion channels built of five heteromeric protein subunits, abundantly expressed in organisms containing a nervous system (Olsen and Sieghart, 2008), with GABA being the endogenous ligand. $GABA_A$ receptor activation leads to hyperpolarisation and inhibition of neurotransmission, thereby, depending on the location of the respective receptors in the central nervous system, leading to the different pharmacological effects seen with benzodiazepines. Sixteen different $GABA_A$ receptors, comprising seven distinct subunit families (α 1–6, β 1–3, γ 1–3, ρ, δ, ε and θ), have been described in humans, with the majority consisting of two α subunits, two β subunits and one γ subunit. The respective isoforms are dispersed within specific regions of the nervous system, thereby accounting for the various pharmacological effects of benzodiazepines. Benzodiazepines do not activate the targeted receptor directly, but increase the frequency of GABA-activated channel-opening by binding as positive allosteric modulators, hence increasing channel conductance (Chebib and Johnston, 2000). This mechanism leads to increasing efficacy of GABA at the $GABA_A$ receptors, resulting in the anxiolytic, anticonvulsant, muscle relaxant, hypnotic and sedative properties of benzodiazepines. The binding site of benzodiazepines is at the interface of an α and a γ subunit. Receptors carrying a γ2 subunit are more sensitive to benzodiazepines than those equipped with a γ1 subunit (Olsen and Sieghart, 2008).

The overall receptor affinity of benzodiazepines is highly dependent on the α subunit. For example, some $GABA_A$ receptors containing an α4 or α6 subunit with β and γ2 do not bind traditional benzodiazepines (Archer and Sternbach, 1968). In addition, a receptor carrying an α1 subunit is said to be linked to the addictive properties of benzodiazepines (Tan et al., 2011). Benzodiazepines can also interact with a structure known as the peripheral benzodiazepine receptor. This translocator protein (TSPO) (18 kDa) is the sole target for Ro5-4864 (also known as 4-chlorodiazepam), a compound that has been shown to induce anxiety and convulsions in rats; this compound recently became available on the internet drug market, and was formally notified in 2016 (EMCDDA, 2017; Pellow and File, 1986). Binding affinities of Ro5-4864, however, are distinctively different among various species (Gavish et al., 1999). Binding to the TSPO can result in anxiolytic effects, without the sedative side effects associated with benzodiazepines (Rupprecht et al., 2009). However, the common pharmacological effects exhibited by benzodiazepines can be explained by binding to $GABA_A$ receptors.

Information about receptor affinities and subtype specificity of new benzodiazepines – if data are available at all – is scarce. Thus, it is impossible to predict with certainty the pharmacological effects of newly emerging substances. There is, however, a high probability that the effects on humans are similar to those of therapeutically used benzodiazepines, with benzodiazepine antagonist flumazenil being capable of reversing those effects. A paper published by Sanna et al. (1999) showed that the anxiolytic effects of etizolam outweighed its sedative properties, which is probably caused by a lower intrinsic activity at $GABA_A$ receptors containing an α1 subunit.

Pharmacodynamics

In general, new benzodiazepines show the same range of effects as benzodiazepines marketed as medicinal drugs. These are anxiolytic effects, sedation, muscle relaxation, anticonvulsant effects and sleep-inducing effects, mediated by binding as allosteric modulators

to $GABA_A$ receptors (see above). Benzodiazepines can also have amnestic and euphoric/mood-lifting effects. In terms of pharmacodynamics, the main difference between benzodiazepines is their duration of action and their relative potency. In general, benzodiazepines can be categorised as 'short-acting' (duration of action is typically in the range of 1–12 hours, e.g. midazolam and triazolam), 'intermediate-acting' (typically 12–40 hours of action, e.g. lorazepam) or 'long-acting' (duration of action > 40 hours, e.g. diazepam and flubromazepam). This usually corresponds to 'short' (1–24 hours), 'medium' (24–48 hours) and 'long' (> 48 hours) elimination half-lives, although, in some cases, the duration of action can be long in spite of a relatively short elimination half-life. This applies to benzodiazepines that are metabolised to pharmacologically active compounds showing longer elimination half-lives than the parent compound, for example diazepam metabolised to the longer-acting nordiazepam. Moreover, potency varies over a large scale, with the most potent drugs producing strong effects at doses of well below 1 mg (e.g. triazolam and flubromazolam), while, for example, oxazepam produces similar effects at doses of about 20 mg. A further distinction is the onset of action, which can vary depending on the absorption rate and the route of administration.

In vitro studies

A limited number of *in vitro* studies are available on new benzodiazepines, and data are mostly restricted to compounds that were pursued for approval as medicines, such as phenazepam and etizolam.

In vitro, phenazepam and its metabolite 3-hydroxyphenazepam potentiate GABA responses with EC_{50} values of 6.1 nM and 10.3 nM respectively, compared with the value of 13.5 nM for diazepam (Kopanitsa et al., 2001, 2002).

In isolated neurons, etizolam behaved as a full benzodiazepine receptor agonist, similar to nitrazepam and diazepam (Yakushiji et al., 1989).

In 2018, on the basis of published binding values to $GABA_A$ receptors, a quantitative structure–activity relationship (QSAR) model to predict $GABA_A$ receptor binding was suggested (Waters et al., 2018), which might be helpful to roughly estimate the binding activity of newly emerging compounds.

Animal studies

Animal studies are available for a limited number of new benzodiazepines, among them 4'-chlorodiazepam – with a focus on potentially neuroprotective properties (Leonelli et al., 2005; Mills et al., 2008) – and etizolam (Woolverton and Nader, 1995) and phenazepam (Molodavkin et al., 1996, 2003), which were compared with already well-characterised benzodiazepines.

In laboratory animals, etizolam induced muscle relaxation, reduced conflict behaviour and had anticonvulsive activity (Johnson and Funderburk, 1978; Tahara et al., 1978). Depending on the parameter and the animal studied, etizolam has been reported to be approximately as active, and up to six times as active, as diazepam (Fukuda and Tsumagari, 1983).

The sedative, tranquillising and muscle relaxant effects of flualprazolam in mice were discussed in a patent by The Upjohn Company (Hester, 1976). Flualprazolam was found to be more potent than alprazolam in all of the tests conducted. The central nervous system depressant activity of flualprazolam was further explored in a study of a series of triazolobenzodiazepine compounds (Hester et al., 1971). The authors reported that compounds substituted with chlorine (i.e. triazolam) or fluorine (i.e. flualprazolam) at the *ortho*-position of the phenyl ring attached to the benzodiazepine moiety were found to exhibit dramatically enhanced activity, compared with the unsubstituted compound (i.e. alprazolam).

Human studies

Human studies are conducted for drug candidates only during clinical evaluation, for example etizolam (Bertolino et al., 1989; Bramanti et al., 1990; Casacchia et al., 1990; De Candia et al., 2009; Fukami et al., 2010; Pariante et al., 1989; Savoldi et al., 1990), which was shown to be similarly as effective as other benzodiazepines that were authorised as medicines at the time, such as alprazolam.

The results of clinical trials showed that etizolam produced significant improvements in chronic anxiety, phobic ideas, associated depressive symptoms and episodic anxiety, and was significantly more effective than placebo (Casacchia et al., 1990; Savoldi et al., 1990). In a double-blind study among patients with generalised anxiety disorders associated with depressive symptoms, the effectiveness and tolerability of etizolam and alprazolam were compared. The results showed that both drugs had marked anxiolytic and antidepressive activity and that there was no significant difference between the two drugs in terms of overall anxiolytic effectiveness (Pariante et al., 1989).

Pharmacokinetics

Information on the pharmacokinetics of new benzodiazepines is limited. Studies in humans were carried out for phenazepam (Zherdev et al., 1982) and etizolam (Fracasso et al., 1991).

In humans, etizolam is a short-acting benzodiazepine. In a study of single- and multiple-dose pharmacokinetics of etizolam in healthy subjects, the maximum plasma concentration (C_{max}) was reached within 0.5–2 hours in all subjects after a single oral administration of 0.5 mg of etizolam. The mean elimination half-life averaged 3.4 hours (Fracasso et al., 1991).

Metabolism

The metabolism of new benzodiazepines generally does not differ from that of 'traditional' benzodiazepines. Most compounds undergo extensive phase I metabolic transformations, mainly mediated by the cytochrome P450 (CYP) enzyme family (UNODC, 1997; Xu et al., 2005). The compounds are predominantly metabolised by CYP3A4 enzymes. To a lesser extent, CYP3A5, CYP2B6, CYP2C18 and CYP2C19 enzymes are also involved in the metabolism of benzodiazepines (Fukasawa et al., 2007; Kilicarslan et al., 2001; Mizuno et al., 2009). Glucuronidation and acetylation by uridine 5'-diphosphoglucuronosyltransferase (UGT) and *N*-acetyltransferase 2 (NAT2), respectively, are the major enzymes involved in phase II metabolism of benzodiazepines.

Depending on their subclass, the following metabolic reactions have been reported in the literature for benzodiazepines.

- Phase I.
  - Hydroxylation.
    - 1,4-benzodiazepines: hydroxylation mainly occurs at the C-3 position; the minor site is C-4' (Breimer, 1979).
    - Imidazo and triazolo compounds: hydroxylation will mainly occur at the methyl group of the annealed heterocycle or the C-4 position (Breimer, 1979; Gorski et al., 1999; Kitagawa et al., 1979).
  - Dealkylation.
    - 1,4-benzodiazepines carrying an alkyl moiety at N-1 are often prone to dealkylation (Schwartz et al., 1965).
  - Reduction of the nitro moiety.
    - The nitro moiety of 1,4-benzodiazepines (at the C-7 position) or triazolobenzodiazepines (at the C-8 position) is reduced to the amine group (Coller et al., 1999; Mattila and Larni, 1980).
- Phase II.
  - O-Glucuronidation.
    - In urine, benzodiazepines carrying a hydroxyl moiety (or the hydroxylated metabolites of benzodiazepines) are excreted as conjugates of glucuronic acid. In the case of, for example, lorazepam and oxazepam, glucuronidation can be almost complete (Greenblatt, 1981; Hyland et al., 2009).
  - N-Glucuronidation.
    - The imidazo-benzodiazepine midazolam and triazolobenzodiazepines are partly excreted as *N*-glucuronides of the parent compound. In the case of midazolam, the conjugation occurs at the N-2 position (Hyland et al., 2009).
  - Acetylation.
    - The amine function of reduced nitro (triazolo-) benzodiazepines can be acetylated metabolically.

The metabolites (*in vivo* and *in vitro*) observed for the benzodiazepines discussed in this review are summarised in Table 1 on page 8. Except for adinazolam (Fraser et al., 1993; Lahti et al., 1983), etizolam (Fracasso et al., 1991; Nakamae et al., 2008) and phenazepam (Maskell et al., 2012; Zherdev et al., 1982), all data published on the metabolism of those compounds result from studies performed as a reaction to the compounds emerging on the drug market.

There are limited data on the specific enzymes involved in the metabolism of new benzodiazepines. In a study from 2017, Noble et al. found that CYP3A4 and CYP3A5 accounted for the formation of the flubromazolam metabolites α-hydroxyflubromazolam, 4-hydroxyflubromazolam and α,4-dihydroxyflubromazolam. 'Classical' triazolo-/imidazobenzodiazepines such as alprazolam, triazolam and midazolam are also hydroxylated at the α- and 4-positions by enzymes of the CYP3A family (Masica et al., 2004). Vikingsson et al. (2017) suggested that the reduction of the nitro moiety in meclonazepam was catalysed by CYP3A4 and that the conjugation leads to 7-acetamidomeclonazepam by NAT2.

Most of the phase I metabolites of benzodiazepines are pharmacologically active. Exceptions are found with the amino metabolites of nitrobenzodiazepines such as the 7-amino metabolites of clonazepam or nitrazepam.

Some metabolites are also marketed as pharmaceutical products, such as temazepam, nordazepam and oxazepam, the main metabolites of diazepam. Some metabolites of triazolobenzodiazepines, for example the α-OH metabolite in the case of alprazolam and triazolam, show high binding affinities towards $GABA_A$ and are considered to be at least as active as the parent compound (Hester and Von Voigtlander, 1979). On the contrary, 4-OH metabolites generally show a reduced biological activity (Baselt, 2011). Significant binding affinity at the $GABA_A$ receptor was also reported for α-OH-midazolam glucuronide, a phase II metabolite of midazolam (Bauer et al., 1995).

Interindividual genetic variability in metabolising enzymes

Several enzymes involved in the metabolism of benzodiazepines are expressed polymorphically. Polymorphisms of CYP3A4 and CYP3A5 do not seem to affect benzodiazepine metabolism; CYP2C19 polymorphisms, however, have been reported to have significant impact, especially for clobazam, etizolam and diazepam (Fukasawa et al., 2005; Goldstein, 2001; Parmeggiani et al., 2004). The elimination half-life of diazepam in people who are CYP2C19 poor metabolisers was twice as long as it was for normal metabolisers (Bertilsson et al., 1989). Regarding phase II metabolism, polymorphisms have been described for NAT2 enzymes mediating N-acetylation of benzodiazepines carrying an amino function (natively or as the result of nitroreduction). Olivera et al. (2007) observed a reduction in the metabolism rate for 7-aminoclonazepam, the major phase I metabolite of clonazepam. Thus, altered metabolism rates due to polymorphisms can increase toxicity and lead to different windows of detection following drug uptake.

Interactions with other drugs

In general, any CYP-inhibiting or -inducing drug can have effects on the metabolisation of benzodiazepines by altering the elimination of the drug. This can result in clinically significant changes of effect size and alteration of the window of detection. CYP3A4 inhibitors such as grapefruit juice, the selective serotonin reuptake inhibitor fluoxetine, azole antifungal drugs and some antibiotics (e.g. erythromycin) drastically reduce benzodiazepine metabolism and subsequent elimination, which can result in adverse effects. Drug accumulation due to decelerated metabolism can influence overall drug toxicity.

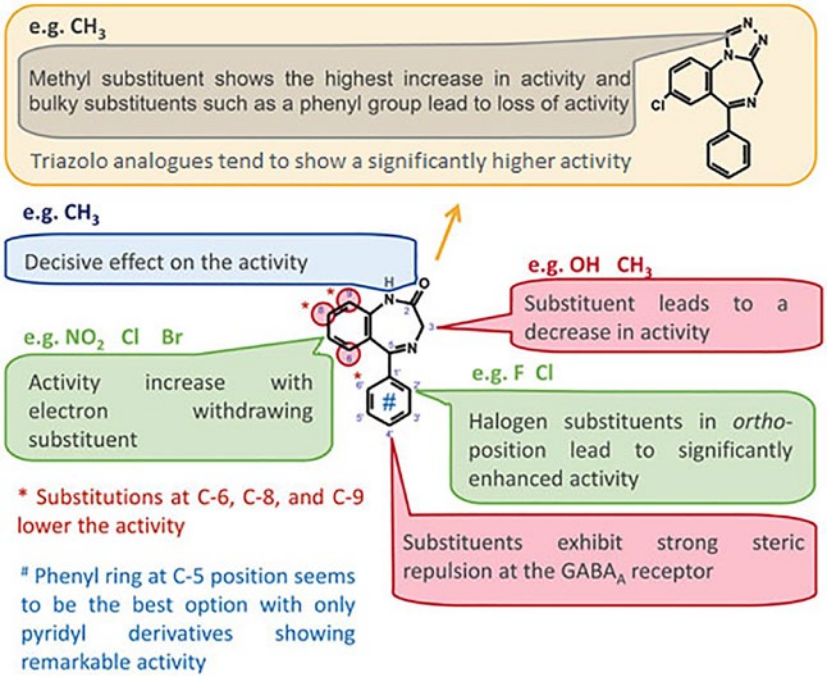
The combined uptake of benzodiazepines with alcohol, opioids or other central nervous system depressants can result in a mutual increase of the respective pharmacological effects, such as increased sedation, impaired motor coordination and respiratory depression. Lethal outcomes after mixed drug consumption have been reported repeatedly (Ellefsen et al., 2017; Koch et al., 2018; Moody, 2004). Although some studies suggest that benzodiazepines and opioids might alter the pharmacokinetic effects of one another, this interaction may not be clinically significant. It seems much more likely that the pharmacodynamic interactions of these drugs are responsible for the mutual reinforcement of effects (Jones et al., 2012).

Structure–activity relationships and drug design

The structure–activity relationships (SARs) of benzodiazepines were first studied by Sternbach et al. and Hester et al. in the late 1960s and 1970s (Sternbach et al., 1968; Hester and Von Voigtlander, 1979). The following SARs are based on their observations for 1,4-benzodiazepines and triazolobenzodiazepines (Figure 5).

- The substituent at C-7 (C-8 for triazolobenzodiazepines) is of paramount importance (Sternbach, 1971). The activity can be increased by an electron-withdrawing substituent (e.g. halogen, CF_3 or NO_2). In the case of 1,4-benzodiazepines, an increase in activity was observed alongside an increase in electronegativity.
- For 1,4-benzodiazepines and triazolobenzodiazepines, a halogen atom (F or Cl) bound to the *ortho*-position of the phenyl ring significantly enhances activity.
- Substitution at N-1 has a decisive effect on the activity of 1,4-benzodiazepines.
- For triazolobenzodiazepines, a methyl substituent at C-1 showed the most significant increase in activity (tested H to n-propyl), and bulky substituents, such as phenyl groups, led to a loss of activity.
- A decrease in activity has been described for 1,4-benzodiazepines with an electron donor present at C-7 and a substituent in the *para*-position of the phenyl ring. Substitutions at C-6, C-8 and C-9 also lowered the activity of the compounds (Sternbach et al., 1968), as does a substituent at C-3.
- The phenyl ring at C-5 appeared to be the best option, with only pyridyl derivatives showing remarkable activity.

FIGURE 5
Structure activity relationship for new benzodiazepines (adapted from Moosmann and Auwärter, 2018)



- According to Waters et al. (2018) substituents on the R4'-position of the phenyl ring show a strong steric repulsion at the $GABA_A$ receptor hence decreasing the activity of the compound.
- A considerably lower activity is also observed by substitution of the carbonyl function at C-2 by a thione (Sternbach et al., 1968).

To predict the biological activity and binding affinity of benzodiazepines becoming available on the 'legal high' market, Waters et al. (2018) used a QSAR approach. Based on a learning set of 69 known benzodiazepines, the model was applied to predict the binding affinities of 22 new benzodiazepines by relating biological activity to structural descriptors. According to the authors, the critical structural elements resulting in high binding affinities were the position of two H-bond acceptors, two aromatic rings and a hydrophobic group. Benzodiazepines with these features showed a greater binding affinity towards the $GABA_A$ receptor than most prescription benzodiazepines. The highest binding affinities (log 1/c) were predicted for the three triazolobenzodiazepines flunitrazolam, clonazolam and flubromazolam, which is in good agreement with the observations made by Hester et al. (1971) that triazolobenzodiazepines are more potent than the corresponding 1,4-benzodiazepines.

QSAR models and SAR evaluations can be helpful to predict the biological activity of new benzodiazepines, but should not be regarded as a replacement for *in vitro* and *in vivo* testing. An overview of the predicted binding affinities to $GABA_A$ receptor and dosages by Waters et al. (2018) can be found in Table 2 on page 20.

Health and social risks

Acute toxicity

Animal data

For many benzodiazepines authorised as medicines, LD_{50} (median lethal dose) values in various species are available. For example, the LD_{50} of orally administered etizolam was reported to be 4 300 mg/kg in mice and 3 550 mg/kg in rats (Tsumagari et al., 1978). The results suggest that etizolam is less toxic than diazepam (LD_{50} values for etizolam were 2–5 times higher than LD_{50} values for diazepam). In another study, the oral LD_{50} for etizolam in mice was reported as 1 780 mg/kg, compared with 690 mg/kg for diazepam (Johnson and Funderburk, 1978). Although lower LD_{50} values were found for other benzodiazepines, there is a consensus that the acute toxicity of benzodiazepines is relatively low compared with that of other drugs of abuse, and can include strong sedation, coma, impaired motor function, hypotension and respiratory depression in a dose-dependent manner.

TABLE 2
Binding values to the $GABA_A$ receptor and dosages of new benzodiazepines

Compound	Predicted binding value (log 1/c)	Common dose (mg) (*)
Flunitrazolam	8.88	0.08–0.15
Clonazolam	8.86	0.2–0.4
Flubromazolam	8.77	0.2–0.4
Etizolam	8.64	1–2
Nifoxipam	8.63	0.5–1
Meclonazepam	8.52	3–6
Fonazepam (desmethyl-flunitrazepam)	8.46	1–2
N-Desalkylflurazepam (norflurazepam)	8.44	5–10
3-Hydroxy-phenazepam	8.42	1–2
Diclazepam (Ro5-3448)	8.39	1–2
Flubromazepam	8.37	4–8
Metizolam (desmethyl-etizolam)	8.34	2–4
Nitrazolam	8.34	1–2
Bromazolam	8.25	1–3
Phenazepam	8.12	1–2
Deschloroetizolam	7.96	4–6
4'-Chlorodiazepam (Ro5-4864)	7.88	n.g.
Cloniprazepam	7.83	1–2
Pyrazolam	7.79	2–3
Adinazolam	7.18	15–30
Flutazolam	6.83	5–10
Ro7-4065 (difludiazepam)	n.t.	n.g.
Flualprazolam	n.t.	0.25–0.5
Fluclotizolam	n.t.	0.25–0.5
Thionordazepam	n.t.	n.g.

(*) Data from https://tripsit.me/ (October 2019).
NB: n.g., not given; n.t., not tested.

Human data

There are numerous reports on benzodiazepine-associated fatalities, although monointoxications as the cause of death are rare. In most cases, the lethal outcome is attributed to multiple drug toxicity, and very often alcohol and opioids/opiates are involved.

Some publications and reports suggest that the use of new benzodiazepines has become an important issue in some countries.

According to the report on drug-related deaths in Scotland in 2019 by the National Records of Scotland, 'street' benzodiazepines (such as etizolam) were implicated in, or potentially contributed to, 814 of the 1 264 drug-related deaths in 2019 (64 % of all drug-related deaths that year). Etizolam has reportedly become the predominant benzodiazepine abused in the illicit drug market across Scotland. In 2019, etizolam was implicated in, or potentially contributed to, the cause of 752 deaths in Scotland (compared with 548 in 2018, 299 in 2017 and 223 in 2016) (National Records of Scotland, 2020).

In a study of 33 post-mortem cases in Sweden and Finland that were positive for flualprazolam, the cause of death was fatal poisoning by a substance in 23 (70 %) cases, and flualprazolam was one of the substances implicated in the fatal poisoning in 13 cases (40 %). In two poisoning cases, flualprazolam was the only intoxicant reported in the cause of death. In these cases, the concentration of flualprazolam was 19 and 21 ng/g respectively (Kriikku et al., 2020). Flualprazolam was also detected in 197 blood samples from medicolegal death investigations

and driving under the influence of drugs (DUID) cases reported between August 2019 and February 2020 in the United States (Papsun et al., 2021). A total of 171 cases were samples collected during medicolegal death (i.e. post-mortem) investigations and 22 were DUID investigations; four cases were submitted under unknown circumstances. In post-mortem cases with quantitative results (n = 167), the mean (± standard deviation (SD)) flualprazolam concentration was 20 (± 63) ng/ml, the median concentration was 8.2 ng/ml, and the range of concentrations was 2.0–620 ng/ml. Flualprazolam was commonly (83 % of the time) found in combination with opioids (e.g. fentanyl).

Chronic toxicity

Animal data

The available data on genotoxicity and carcinogenicity of benzodiazepines and benzodiazepine analogues have been reviewed by Brambilla et al. (2007). They collected data on 51 drugs, 41 of which are still on the market. They were not able to retrieve genotoxicity or carcinogenicity data for 12 drugs, but they found at least one test result for the remaining 39. Of these, nine tested positive in at least one genotoxicity assay, eight tested positive in at least one carcinogenicity assay, and five gave a positive result in at least one genotoxicity assay and in at least one carcinogenicity assay. None of the 11 drugs without positive results in these tests showed carcinogenic activity in mice and/or rats or in other species. Concerning the predictivity of genetic toxicology findings for the result(s) of long-term carcinogenesis assays, 18 drugs had both genotoxicity and carcinogenicity data; of these, 11 were neither genotoxic nor carcinogenic, two were carcinogenic in at least one sex of mice or rats but tested negative in genotoxicity assays, and five gave a positive response in at least one genotoxicity assay and in at least one carcinogenicity assay. The authors conclude that only a small proportion of the marketed benzodiazepines/benzodiazepine analogues (8 out of 41) had all the data required by current guidelines for the testing of pharmaceuticals. No such data are available for new benzodiazepines. However, it seems likely that some of these compounds share the genotoxic/carcinogenic properties of the investigated compounds.

Human data

Some, but not all, benzodiazepines seem to have teratogenic effects. Congenital abnormalities, mainly cleft lip/palate and anal atresia, were reported to be associated with benzodiazepine use during the first 3 months of pregnancy (Dolovich et al., 1998; Iqbal et al., 2002). Consequently, the US Food and Drug Administration (FDA) classified some benzodiazepines (alprazolam, chlordiazepoxide, clonazepam, diazepam and lorazepam) as category D (i.e. positive evidence of risk exists, but benefits from use might outweigh the risk), while others were classified as contraindicated in pregnancy (estazolam, flurazepam, quazepam, temazepam and triazolam). In addition, neonatal withdrawal reactions have been described subsequent to pregnant women taking benzodiazepine medication during the last weeks of pregnancy, and neonatal exposure to benzodiazepines during breastfeeding might lead to central nervous system depression in infants (Iqbal et al., 2002). There are no human data on new benzodiazepines on these issues so far, but it can be assumed that some of these compounds have teratogenic effects and all of them can produce a neonatal withdrawal reaction.

Long-term benzodiazepine use may lead to cognitive impairment. Available information from the scientific literature suggests that long-term benzodiazepine users were significantly impaired, compared with controls, in areas such as visuospatial ability, speed of processing, memory, concentration and verbal learning (Barker et al., 2004). No such data are available for new benzodiazepines. However, it seems likely that some of these compounds might impair cognitive functions when used long term.

Managing poisoning (including mixed intoxications with opioids)

Unlike most of the other groups of NPS, new benzodiazepines (and opioids) were not included in the Novel Psychoactive Treatment: UK Network (NEPTUNE) guidelines on the clinical management of acute and chronic harms of club drugs and NPS. The main acute problems occurring after high doses of opioids or benzodiazepines are loss of consciousness and respiratory depression. Mono-intoxications with benzodiazepines frequently cause unconsciousness, but respiratory depression is much less pronounced than with opioids and mortality is relatively low (Penninga et al., 2016). For both groups of drugs, there are specific antidotes that reversibly and competitively block the binding site at the receptors, for example flumazenil for benzodiazepines and naloxone for opioids. Both antidotes are often used for diagnostic reasons in emergency cases with suspected benzodiazepine or opioid toxicity (spontaneous reversal of the symptoms after application of the antidote is a clear

reference to the type of intoxicating agent). However, the use of flumazenil in the treatment of patients intoxicated with benzodiazepines is controversial because of the adverse events (most commonly supraventricular arrhythmia and convulsions) associated with this treatment, which have been repeatedly reported (Penninga et al., 2016), and are potentially caused by conditions known to be typical contraindications for flumazenil administration (among them co-ingestion of proconvulsive drugs, seizure disorder, sedative-hypnotic drug withdrawal and intoxicated patients with myoclonic jerking or seizure activity). Usually, the clinical management of benzodiazepine overdoses can be achieved by mechanical ventilation, monitoring and (if needed) stabilisation of cardiovascular functions.

Psychological and behavioural effects

The psychological and behavioural effects of new benzodiazepines can be assumed to be similar to the known effects of benzodiazepines authorised as medicines. 'Positive', therapeutically useful effects include anxiolysis (Starcevic, 2014), sleep-inducing, amnestic, anticonvulsant/muscle-relaxing and mild antidepressant effects. On the other hand, particularly among elderly patients, muscle relaxation and psychomotoric impairment can lead to a greater risk of falling and impaired cognitive functioning, and there is some evidence that prolonged benzodiazepine use is associated with a greater risk of developing dementia (Penninkilampi and Eslick, 2018). Although a rather rare complication, 'paradoxical reactions' after the intake of benzodiazepines have been described and can be of concern (Van Der Bijl and Roelofse, 1991). These include disinhibition, excitement, convulsions, aggressive behaviour and agitated toxic psychosis/ hallucinations. Such unusual reactions seem to occur more often among children, young adults and elderly people. In addition, there is the well-known potential for abuse and the development of dependence (Baldwin et al., 2013; Dell'Osso and Lader, 2013), which can lead to severe, and even life-threatening, withdrawal symptoms.

Dependence and abuse potential

Animal data

Drug discrimination studies were performed for many classical benzodiazepines. A common finding is that benzodiazepines fully substitute for barbiturates or other benzodiazepines, and the development of tolerance and withdrawal symptoms occurs after repeated administration. Although there are no data on new benzodiazepines, similar properties can be assumed for these compounds.

In a drug discrimination study with rhesus monkeys, etizolam, like diazepam, fully substituted for pentobarbital (Woolverton and Nader, 1995). The effective dose that gave a 50 % response (ED_{50}) was 1.2 mg/kg for etizolam and 0.8 mg/kg for diazepam.

Human data

Clinical studies with humans also demonstrated that these drugs maintain self-administration behaviour (Cole, 1990; Griffiths and Weerts, 1997; Woods et al., 1987, 1992). However, in comparison to the self-administration responses produced by highly dependence-producing drugs such as opioids/opiates or cocaine, benzodiazepines are generally regarded as weak reinforcers (Jones et al., 2012).

Preclinical evidence exists that benzodiazepines increase the rewarding and reinforcing effects of opioids (Panlilio et al., 2005; Walker and Ettenberg, 2005), probably by amplifying the μ-opioid receptor agonist effects of opioids. This might provide an explanation for the widespread co-use of benzodiazepines among opioid-dependent patients (Jones et al., 2012).

Few case reports of etizolam dependence have been reported in the scientific literature (Gupta and Garg, 2014; Nishii et al.; 2014). The withdrawal symptoms reported for etizolam are characteristic of those reported for benzodiazepine withdrawal (palpitations, impaired sleep, agitation, tremors).

Effects on the ability to drive and operate machines

Because of the pharmacological effects of benzodiazepines, the ability to drive and operate machinery can be massively impaired under the acute influence of benzodiazepines, depending on the dosage. The main potential problems are compromised attention, memory storage and reaction times (Stone et al., 2015). The sedative effects also often lead to drowsiness and/or dizziness; furthermore, confusion and impaired judgement may occur. This is aggravated by the co-consumption of alcohol (Downey et al., 2017) or other central nervous system depressants. On the other hand, the intake of a low dose of a benzodiazepine does not necessarily lead to relevant impairment, as recently shown for etizolam (up to 1 mg) by Busardo et al. (2019).

Regarding impairment after continued chronic benzodiazepine intake (medication or abuse), tolerance for the acutely impairing effects can be assumed. However, in this case, fitness to drive and to operate machines might be impaired as a result of neurocognitive deficits potentially caused by long-term use. The high potency of some of the compounds and their varying half-lives can lead to unpredictable accumulation and 'hangover' effects, particularly among users of new benzodiazepines, who tend to use relatively large doses.

Social risks

The social risks connected to the long-term use of benzodiazepines in general include, but are not limited to, development of lethargy and lack of motivation, depression, and dependence (Baldwin et al., 2013; Dell'Osso and Lader, 2013). These may negatively affect a person's education, career, family relationships or other personal and social relationships, and may result in marginalisation.

Along with ethanol, sedative-hypnotic medicines such as benzodiazepines are one of the groups of substances most commonly used to commit drug facilitated crimes, in particular, drug-facilitated sexual assaults and/or are identified in biological samples taken from sexual assault victims (Xiang et al., 2018). Concerns over the use of flunitrazepam (Rohypnol) to commit drug-facilitated sexual assault were a major factor in its withdrawal from the European market (in this case, the risk outweighed the therapeutic benefit).

Between 2019 and the first half of 2020, the EMCDDA has received a limited but concerning number of reports indicating that new benzodiazepines appear to have been used as incapacitating agents in cases of rape and sexual assault. The substances mentioned in these reports were clozapine, clonazolam, diclazepam, flubromazolam and/or flualprazolam, all of which are monitored as NPS by the EMCDDA. In some cases, liquids containing one or more of these substances were seized in connection to the cases. Some of these cases appear to have occurred in clusters around a particular geographical location. Case reports of drug-facilitated crimes involving the apparent use of new benzodiazepines have also been reported in the literature (Qian et al., 2020; Xiang et al., 2018).

The use of benzodiazepines to commit drug-facilitated crimes could cause serious individual and public health issues, as well as serious social harms.

Extent and patterns of use, availability and potential for diffusion

Prevalence of use

Characteristics of user groups

User groups of new benzodiazepines are not well characterised. Based on their effects and their similarities to prescription benzodiazepines, it can be assumed that new benzodiazepines are used to help with insomnia and to reduce stress ('self-medication'), by people who experience benzodiazepine dependence, in polydrug-use settings to counteract the effects of stimulants or hallucinogens, to enhance and prolong the 'high' of other drugs (usually opioids), and/or to alleviate withdrawal effects caused by other dependence-producing drugs. People using fake medicines containing new benzodiazepines will be unaware that they are using them. It also appears that there is interest in new benzodiazepines by some psychonauts, as is the case with other NPS. Benzodiazepines appear to be perceived by users as relatively safe and socially acceptable (Peters et al., 2007).

Use by vulnerable groups

Benzodiazepines are frequently used by people who use opioids, including high-risk opioid users (EMCDDA, 2018). In some cases, new benzodiazepines have become a relatively low-cost alternative to prescription benzodiazepines available on the illicit market. The misuse of benzodiazepines contributes to increased morbidity and mortality among high-risk opioid users.

New benzodiazepines might also be considered appealing by prisoners (Ford and Berg, 2018), although the available data suggest that synthetic cannabinoids are more popular in this environment. A study of French male prisoners found that 37 % were chronic benzodiazepine misusers and that many high-risk opioid users switched from illicit opioids to benzodiazepines when incarcerated, either to make their incarceration bearable or to cope with withdrawal symptoms (Expertise Collective, 2012).

Patterns of use

When used as a 'standby medication' by users of stimulants or hallucinogens, the frequency of uptake can be estimated to be rather low. However, when used for other purposes, such as 'self-medication' of insomnia or stress reduction, more regular use seems likely. In polydrug-use settings including opioid users, frequent and excessive use of benzodiazepines is very common to enhance or prolong the effects of other drugs or to avoid withdrawal symptoms.

Route of administration

Most prescription benzodiazepines are administered orally or intravenously. Less common routes of administration are intramuscular injection and rectal administration. For new benzodiazepines, oral administration seems to be the most common route of administration, although injecting, smoking/vaping and snorting have also been reported.

Dosage

'Typical' doses for new benzodiazepines vary over a wide range and depend on factors such as binding affinity to the $GABA_A$ receptor, protein binding and their ability to cross the blood–brain barrier (El Balkhi et al., 2020). Since most of the compounds sold on the 'legal high' market have never undergone pharmacological studies and clinical trials, the 'common' doses are estimated and discussed on internet platforms such as TripSit (https://tripsit.me/). For the benzodiazepines discussed in this report, the dosage information, according to TripSit, can be found in Table 2 on page 20, with individual doses ranging from as low as 0.08 mg for flunitrazolam to 30 mg for adinazolam.

Availability, supply and involvement of organised crime

Production

The available information suggests that most new benzodiazepines, like many other NPS, are synthesised in chemical and pharmaceutical laboratories in China; they are shipped as bulk powders to Europe. Once in Europe, the powders are processed into finished products; typically, these are tablets, but capsules, blotters and e-liquids for vaping have also been reported. Some of the new benzodiazepines, particularly etizolam, may be sourced from companies in India, typically as finished medicinal products (Figure 6).

Police Scotland reported that large-scale illicit manufacturing of benzodiazepine tablets, predominantly containing etizolam, occurred between 2017 and 2018 in Scotland and that crime groups are increasingly becoming involved in the production of these tablets. The huge demand for the tablets on the illicit drug market has resulted in several large-scale producers setting up illicit production sites in the west of Scotland. The detection of these production sites illustrates that professional equipment (such as multi-station rotary pill presses; Figure 7) is being used by illicit manufacturers to enable quick and efficient large-scale production. During 2017, police seized 1.67 million tablets containing etizolam at

FIGURE 6
Tablets containing etizolam that originated from India, seized by Spanish customs in February 2019

Photo: Spanish customs.

FIGURE 7
Rotary pill press seized at an illicit production site

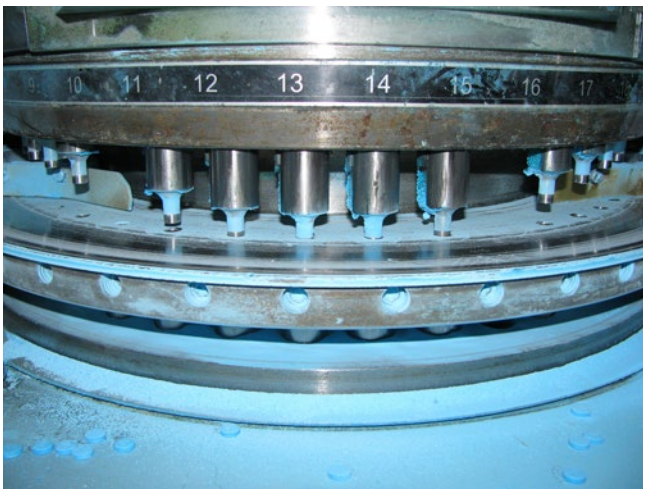


Photo: Police Scotland.

one such production site. Their estimated street value was EUR 1.9 million. The etizolam was sourced as a bulk powder from China, diluted, mixed with blue dye and then pressed into tablets using an industrial tablet press. The amount of etizolam in each tablet was around 0.8 mg, meaning that the amount of active ingredient needed to produce the number of tablets seized at the time of the police raid was less than 1.5 kg (EMCDDA and Europol, 2019).

Trafficking

There are indications that benzodiazepines are ordered from legitimate chemical and pharmaceutical companies in China, which ship the products typically as powders by mail and courier services to retailers and individuals in Europe. As with other NPS, in some cases consignments containing new benzodiazepines are misdeclared as other goods or concealed using methods similar to those used for established drugs. Furthermore, finished products might be ordered from unlicensed online pharmacies based in India.

Distribution among users, so called 'social supply', also seems to play an important role, in particular for opioid and poly-drug users.

Internet markets

New benzodiazepines may be sold on the surface web and the darknet (EMCDDA and Europol, 2017). There are a variety of internet retailers offering 'research chemicals' and 'legal highs' containing new benzodiazepines.

Falsified (fake) medicines

Initially, many new benzodiazepines were sold under their own names and advertised as 'research chemicals' and 'legal highs'. While this continues to be the case, increasingly these substances are also being used to produce fake benzodiazepine medicines, particularly fake diazepam tablets (Valium) and fake alprazolam tablets (Xanax), which are then sold on the illicit drug market. In some cases, the fake tablets are packaged in blister packs resembling those of the legitimate products (Figure 8).

In the past few years, etizolam and flualprazolam, in particular, have played an increasingly important role in the new benzodiazepine market in some parts of Europe, especially in making fake diazepam and alprazolam tablets (EMCDDA, 2019b, 2019c; Nielsen and McAuley, 2020).

FIGURE 8

Fake diazepam tablets packaged in blister packs resembling those of the legitimate products. The tablets were purchased as 'Diazepam Activis' but were found to contain flubromazolam and diazepam on analysis by Wedinos in May 2020

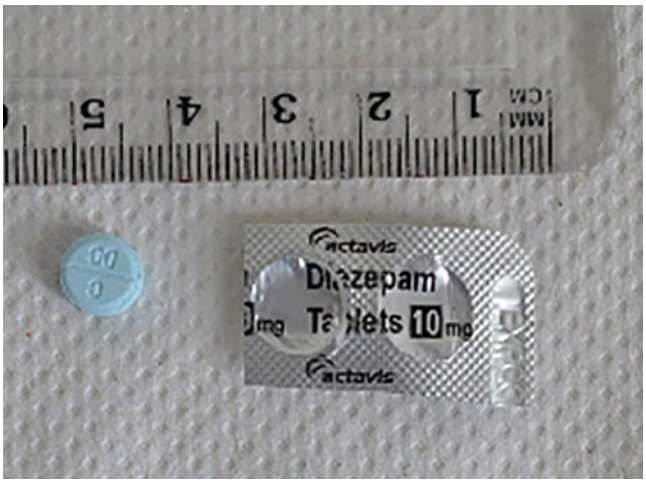


Photo: Wedinos.

European drug-checking services Wedinos, in Wales, United Kingdom, ([9]) and Saferparty, Zurich, Switzerland, ([10]) have reported an increasing number of fake Xanax and Valium tablets containing flualprazolam, flubromazolam, etizolam and other new benzodiazepines in recent years.

In Scotland, criminal groups are known to be involved in the large-scale illicit manufacture and distribution of fake benzodiazepine medicines. Typically made to look like 10-mg diazepam tablets, and known as 'street Valium', these fakes often contain new benzodiazepines. During 2016 and 2017, one such benzodiazepine, etizolam, was reported to have become the predominant substance within the illicit market for benzodiazepines, with 65 % of tablets found containing the substance (EMCDDA and Europol, 2019).

Data from Wedinos, the drug-testing service operated by Public Health Wales in the United Kingdom, suggest that flubromazolam and deschloroetizolam have recently re-emerged on the drug market in the United Kingdom and are being used to make fake diazepam, fake alprazolam, fake temazepam and fake zopiclone (Wedinos, 2020). Some recent poisonings in the United Kingdom have been linked to flubromazolam sold as fake benzodiazepine medicines (Public Health England, 2020).

([9]) Wedinos is a drug-testing service in the United Kingdom operated by Public Health Wales (http://www.wedinos.org/about_us.html).
([10]) Saferparty is a drug-testing service in Zurich, Switzerland (https://www.saferparty.ch/ueber-uns.html).

In a case from Singapore and Malaysia, Erimin 5 tablets contained phenazepam instead of nimetazepam (Lim et al., 2017).

Quality on the market

Typical impurities

Typical impurities have not been analytically assessed so far. It can be assumed that such contaminants would typically originate from synthesis (by-products that were not sufficiently separated during clean-up of the raw material), depending on the chosen route of synthesis.

Contaminants

So far, no typical contaminants have been analytically detected.

New benzodiazepines as adulterants

A recently published study reported an outbreak of adulteration of illicit opioids with new benzodiazepines in Vancouver, Canada, between October 2018 and January 2020 (Laing et al., 2021). The reasons for this type of adulteration are unclear; however, it has been suggested that benzodiazepines may serve to 'heighten' or prolong the 'high' associated with opioid use, may help users to cope with opioid withdrawal symptoms or may control the 'descent phase' of opioid intoxication.

Conclusion

New benzodiazepines have established themselves as a group of NPS. It is likely that new substances belonging to this group will continue to appear, not only to satisfy customers such as 'psychonauts' seeking new drug experiences, but also to circumvent drug tests (e.g. workplace drug testing) and as an alternative to unavailable prescription medicines.

Over the past few years, there has been an increase in the number and availability of new benzodiazepines on the drug market in Europe and, increasingly, elsewhere. As of 28 February 2021, the EMCDDA, through the EU Early Warning System on NPS, is monitoring 30 new benzodiazepines that have appeared on the drug market since the mid 2000s. Of these, more than 80 % were detected for the first time between 2014 and 2020. Despite this relatively large number, the market in Europe is dominated by a handful of substances. In the past few years, etizolam and flualprazolam, in particular, have played an increasingly important role in the new benzodiazepine market in some parts of Europe, especially in making fake diazepam and alprazolam tablets.

Overall, these developments give rise to concerns about both individual and public health for a number of reasons. The pharmacology and toxicology of new benzodiazepines is largely unknown, and the risks associated with their use may be higher than those associated with the use of authorised benzodiazepine medicines. In addition, the very nature of unregulated markets means that these risks may be amplified because users cannot be certain of the dose used. In some cases, users may not be aware that they are using these substances; therefore, they might be at higher risk of severe poisoning, particularly if taken in combination with other central nervous system depressant drugs, such as alcohol and opioids. Of particular concern is the growing use of new benzodiazepines to make falsified (fake) tablets of commonly prescribed benzodiazepine medicines, such as diazepam (Valium) and alprazolam (Xanax), and the involvement of criminal groups in producing such tablets. In some cases, the fake tablets are packaged in blister packs resembling those of the legitimate products, which makes it more difficult for consumers to spot the fakes. Serious adverse events, such as severe poisoning, involving such fake medicines have been reported in Europe. Other risks might be related to the potential presence of adulterants, other substances or synthesis by-products from illicit manufacture and processing.

The reason for the increase in availability of new benzodiazepines in Europe is not entirely clear. In part, the increase mirrors the general increased availability of a range of NPS since the mid 2000s. In addition, given the widespread use of prescription benzodiazepines in society, and their diversion to the illicit drug market, the increase in new benzodiazepines might also be partially related to well-intentioned restrictions in the legal supply of authorised benzodiazepine medicines and the introduction of prescription limits in order to prevent or reduce harms among patients, such as dependence. While this is speculative, some support for this may come from the increasing number of fake benzodiazepine medicines that have been seized on the illicit drug market in the past few years that contain new benzodiazepines.

In future, it can be expected that compounds with high potency and that are easy to synthesise will continue to be introduced into the market. Therefore, further triazolo derivatives can be expected as newly emerging candidates. In addition, there might be efforts to circumvent the (chemical) definition of generic approaches, as has been seen for other NPS.

The ongoing COVID-19 pandemic and related response measures may affect the existing benzodiazepine drug markets in unpredictable ways. Such effects may extend to changes in use and patterns of use of benzodiazepines, including a possible increase in prescriptions in order to treat insomnia and anxiety related to the pandemic. Ultimately, such changes may lead to a greater demand for new benzodiazepines, such as individuals seeking out new benzodiazepines to self-medicate, or inadvertently using new benzodiazepines through the use of fake medicines. New benzodiazepines may also be mis-sold as, or used to adulterate, other illicit drugs – such as the recent phenomenon of 'benzo dope' in Canada, where new benzodiazepines have been used to adulterate the illicit opioids supply.

Given the growing complexity of the market in new benzodiazepines and its strong links with the broader illicit drug market, particularly the large demand for benzodiazepine medicines such as alprazolam and diazepam, there is a need to ensure that we continue to strengthen the ability to detect, assess and respond to existing and new threats in a timely and effective way, to prevent or reduce the public health and social harms caused by this group of substances.

References

Ameline, A., Richeval, C., Gaulier, J. M., Raul, J. S. and Kintz, P. (2019), 'Detection of the designer benzodiazepine flunitrazolam in urine and preliminary data on its metabolism', *Drug Testing and Analysis*, Vol. 11, No 2, pp. 223–229 (https://doi.org/10.1002/dta.2480).

Andersson, M. and Kjellgren, A. (2017), 'The slippery slope of flubromazolam: experiences of a novel psychoactive benzodiazepine as discussed on a Swedish online forum', *Nordisk Alkohol & Narkotikatidskrift*, Vol. 34, No 3, pp. 217–229 (https://doi.org/10.1177/1455072517706304).

Archer, G. A. and Sternbach, L. H. (1968), 'Chemistry of benzodiazepines', *Chemical Reviews*, Vol. 68, No 6, pp. 747–784 (https://doi.org/10.1021/cr60256a004).

Babbini, M., Gaiardi, M. and Bartoletti, M. (1979), 'Anxiolytic versus sedative properties in the benzodiazepines series: differences in structure–activity relationships', *Life Sciences*, Vol. 25, No 1, pp. 15–22 (https://doi.org/10.1016/0024-3205(79)90484-3).

Bäckberg, M., Pettersson Bergstrand, M., Beck, O. and Helander, A. (2019), 'Occurrence and time course of NPS benzodiazepines in Sweden – results from intoxication cases in the STRIDA project', *Clinical Toxicology (Phila)*, Vol. 57, No 3, pp. 203–212 (https://doi.org/10.1080/15563650.2018.1506130).

Baldwin, D. S., Aitchison, K., Bateson, A., Curran, H. V., Davies, S., Leonard, B., Nutt, D. J., Stephens, D. N. and Wilson, S. (2013), 'Benzodiazepines: risks and benefits. A reconsideration', *Journal of Psychopharmacology*, Vol. 27, No 11, pp. 967–971 (https://doi.org/10.1177/0269881113503509).

Barker, M. J., Greenwood, K. M., Jackson, M. and Crowe, S. F. (2004), 'Cognitive effects of long-term benzodiazepine use: a meta-analysis', *CNS Drugs*, Vol. 18, No 1, pp. 37–48 (https://doi.org/10.2165/00023210-200418010-00004).

Baselt, R. C. (2011), *Disposition of Toxic Drugs and Chemicals in Man*, 9th edition, Biomedical Publications, Seal Beach, CA.

Bauer, T. M., Ritz, R., Haberthür, C., Haefeli, W., Scollo-Lavizzari, G., Ha, H., Hunkeler, W. and Sleight, A. J. (1995), 'Prolonged sedation due to accumulation of conjugated metabolites of midazolam', *The Lancet*, Vol. 346, No 8968, pp. 145–147 (https://doi.org/10.1016/s0140-6736(95)91209-6).

Bertilsson, L., Henthorn, T. K., Sanz, E., Tybring, G., Säwe, J. and Villén, T. (1989), 'Importance of genetic factors in the regulation of diazepam metabolism: relationship to S-mephenytoin, but not debrisoquin, hydroxylation phenotype', *Clinical Pharmacology and Therapeutics*, Vol. 45, No 4, pp. 348–355 (https://doi.org/10.1038/clpt.1989.40).

Bertolino, A., Mastucci, E., Porro, V., Corfiati, L., Palermo, M., Ecari, U. and Ceccarelli, G. (1989), 'Etizolam in the treatment of generalized anxiety disorder: a controlled clinical trial', *The Journal of International Medical Research*, Vol. 17, No 5, pp. 455–460 (https://doi.org/10.1177/030006058901700507).

Bradley, C. M. and Nicholson, A. N. (1984), 'Activity of the chloro- and triazolo-benzodiazepines. Behavioural studies in the monkey (Macaca mulatta)', *Neuropharmacology*, Vol. 23, No 3, pp. 327–331 (https://doi.org/10.1016/0028-3908(84)90195-3).

Bramanti, P., Ricci, R. M., Rifici, C., Ecari, U. and Di Perri, R. (1990), 'Etizolam: a controlled study versus alprazolam in the treatment of generalized anxiety disorder with minor associated depressive symptoms', *Current Therapeutic Research*, Vol. 48, No 2, pp. 369–377.

Brambilla, G., Carrozzino, R. and Martelli, A. (2007), 'Genotoxicity and carcinogenicity studies of benzodiazepines', *Pharmacological Research*, Vol. 56, No 6, pp. 443–458 (https://doi.org/10.1016/j.phrs.2007.08.006).

Breimer, D. D. (1979), 'Pharmacokinetics and metabolism of various benzodiazepines used as hypnotics', *British Journal of Clinical Pharmacology*, Vol. 8, pp. 7S–13S (https://doi.org/10.1111/j.1365-2125.1979.tb00449.x).

Breimer, D. D. and Jochemsen, R. (1983), 'Clinical pharmacokinetics of hypnotic benzodiazepines: a summary', *British Journal of Clinical Pharmacology*, Vol. 16 (Suppl. 2), pp. 277S–278S (https://doi.org/10.1111/j.1365-2125.1983.tb02300.x).

Busardo, F. P., Di Trana, A., Montanari, E., Mauloni, S., Tagliabracci, A. and Giorgetti, R. (2019), 'Is etizolam a safe medication? Effects on psychomotor perfomance at therapeutic dosages of a newly abused psychoactive substance', *Forensic Science International*, Vol. 301, pp. 137–141 (https://doi.org/10.1016/j.forsciint.2019.05.018).

Carpenter, J. E., Murray, B. P., Dunkley, C., Kazzi, Z. N. and Gittinger, M. H. (2019), 'Designer benzodiazepines: a report of exposures recorded in the National Poison Data System, 2014–2017', *Clinical Toxicology (Phila)*, Vol. 57, No 4, pp. 282–286 (https://doi.org/10.1080/15563650.2018.1510502).

Casacchia, M., Bolino, F. and Ecari, U. (1990), 'Etizolam in the treatment of generalized anxiety disorder: a double-blind study versus placebo', *Current Medical Research and Opinion*, Vol. 12, No 4, pp. 215-223 (https://doi.org/10.1185/03007999009111650).

Center for Forensic Science Research and Education (2021), *Trend Report – Q4 2020: NPS benzodiazepines in the United States* (https://www.npsdiscovery.org/wp-content/uploads/2021/01/2020-Q4_NPS-Benzodiazepines_Trend-Report.pdf).

Chase, G. O. (1976), 'Process for preparing benzodiazepines', US patent No 3996209A (https://patents.google.com/patent/US3996209A/en?oq=US3996209).

Chebib, M. and Johnston, G. A. (2000), 'GABA-Activated ligand gated ion channels: medicinal chemistry and molecular biology', *Journal of Medicinal Chemistry*, Vol. 43, No 8, pp. 1427–1447 (https://doi.org/10.1021/jm9904349).

Cho, M. J., Sethy, V. H. and Haynes, L. C. (1986), 'Sequentially labile water-soluble prodrugs of alprazolam', *Journal of Medicinal Chemistry*, Vol. 29, No 8, pp. 1346–1350 (https://doi.org/10.1021/jm00158a004).

Clayton, T., Poe, M. M., Rallapalli, S., Biawat, P., Savić, M. M., Rowlett, J. K., Gallos, G., Emala, C. W., Kaczorowski, C. C., Stafford, D. C., Arnold, L. A. and Cook, J. M. (2015), 'A review of the updated pharmacophore for the alpha 5 GABA(a) benzodiazepine receptor model', *International Journal of Medicinal Chemistry*, Vol. 2015, No 430248 (https://doi.org/10.1155/2015/430248).

Coassolo, P., Aubert, C. and Cano, J. P. (1985), 'Plasma determination of 3-methylclonazepam by capillary gas chromatography', *Journal of Chromatography*, Vol. 338, No 2, pp. 347–355 (https://doi.org/10.1016/0378-4347(85)80105-5).

Cole, S. O. (1990), 'Diazepam-induced impairment of a go–no go successive discrimination', *Behavioral and Neural Biology*, Vol. 53, No 3, pp. 371–377 (https://doi.org/10.1016/0163-1047(90)90240-7).

Coller, J. K., Somogyi, A. A. and Bochner, F. (1999), 'Flunitrazepam oxidative metabolism in human liver microsomes: involvement of CYP2C19 and CYP3A4', *Xenobiotica*, Vol. 29, pp. 973–986 (https://doi.org/10.1080/004982599238056).

Cook, J. M., Huang, Q., He, X., Li, X., Yu, J. and Han, D. (2010), 'Anticonvulsant and anxiolytic methods of using receptor subtype selective agents', US patent No 20100130481A1 (https://patents.google.com/patent/US20100130481).

De Candia, M. P., Di Sciascio, G., Durbano, F., Mencacci, C., Rubiera, M., Aguglia, E., Garavini, A., Bersani, G., Di Sotto, A., Placidi, G. and Cesana, B. M. (2009), 'Effects of treatment with etizolam 0.5 mg BID on cognitive performance: a 3-week, multicenter, randomized, double-blind, placebo-controlled, two-treatment, three-period, noninferiority crossover study in patients with anxiety disorder', *Clinical Therapeutics*, Vol. 31, No 12, pp. 2851–2859 (https://doi.org/10.1016/j.clinthera.2009.12.010).

Dell'Osso, B. and Lader, M. (2013), 'Do benzodiazepines still deserve a major role in the treatment of psychiatric disorders? A critical reappraisal', *European Psychiatry*, Vol. 28, No 1, pp. 7–20 (https://doi.org/10.1016/j.eurpsy.2011.11.003).

Dolovich, L. R., Addis, A., Vaillancourt, J. M., Power, J. D., Koren, G. and Einarson, T. R. (1998), 'Benzodiazepine use in pregnancy and major malformations or oral cleft: meta-analysis of cohort and case–control studies', *BMJ*, Vol. 317, No 7162, pp. 839–843 (https://doi.org/10.1136/bmj.317.7162.839).

Downey, L. A., Ford, T. and Hayley, A. (2017), 'Editorial: asleep at the wheel – concerning driving after co-consumption of alcohol and benzodiazepines', *Current Drug Abuse Reviews*, Vol. 10, No 1, pp. 4–5 (https://doi.org/10.2174/1874473710011806110 82250).

El Balkhi, S., Chaslot, M., Picard, N., Dulaurent, S., Delage, M., Mathieu, O. and Saint-Marcoux, F. (2017), 'Characterization and identification of eight designer benzodiazepine metabolites by incubation with human liver microsomes and analysis by a triple quadrupole mass spectrometer', *International Journal of Legal Medicine*, Vol. 131, pp. 979–988 (https://doi.org/10.1007/s00414-017-1541-6).

El Balkhi, S., Monchaud, C., Herault, F., Géniaux, H. and Saint-Marcoux, F. (2020), 'Designer benzodiazepines' pharmacological effects and potencies: how to find the information', *Journal of Psychopharmacology*, Vol. 34, No 9 pp. 1021–1029 (https://doi.org/10.1177/0269881119901096).

Ellefsen, K. N., Taylor, E. A., Simmons, P., Willoughby, V. and Hall, B. J. (2017), 'Multiple drug-toxicity involving novel psychoactive substances, 3-fluorophenmetrazine and U-47700', *Journal of Analytical Toxicology*, Vol. 41, No 9, pp. 765–770 (https://doi.org/10.1093/jat/bkx060).

EMCDDA (European Monitoring Centre for Drugs and Drug Addiction) (2017), *European Drug Report 2017 – Trends and developments*, Publications Office of the European Union, Luxembourg (https://www.emcdda.europa.eu/publications/edr/trends-developments/2017_en).

EMCDDA (2018), *Perspectives on Drugs – The misuse of benzodiazepines among high-risk opioid users in Europe* (http://www.emcdda.europa.eu/publications/pods/benzodiazepines).

EMCDDA (2019a), *EMCDDA operating guidelines for the European Union Early Warning System on new psychoactive substances,* Publications Office of the European Union, Luxembourg (https://www.emcdda.europa.eu/system/files/publications/12213/EWS%20guidelines_final.pdf).

EMCDDA (2019b), *EU Early Warning System Advisory – Flualprazolam: Overview of the situation in Europe*, EU-EWS-RCS-AD-2019-0001, issued 12 March 2019.

EMCDDA (2019c), *EU Early Warning System Briefing – Etizolam: Overview of the situation in Europe,* EU-EWS-RCS-BR-2019-0001, issued 7 March 2019.

EMCDDA (2020), *New Psychoactive Substances – Global markets, glocal threats and the COVID-19 pandemic: An update from the EU Early Warning System (December 2020),* Publications Office of the European Union, Luxembourg (https://www.emcdda.europa.eu/publications/rapid-communication/new-psychoactive-substances-global-markets-glocal-threats-and-covid-19-pandemic_en).

EMCDDA and Europol (2012), *EMCDDA–Europol 2011 annual report on the implementation of Council Decision 2005/387/JHA* (https://www.emcdda.europa.eu/publications/implementation-reports/2011_en).

EMCDDA and Europol (2013), *New Drugs in Europe, 2012: EMCDDA–Europol 2012 annual report on the implementation of Council Decision 2005/387/JHA* (https://www.emcdda.europa.eu/publications/implementation-reports/2012_en).

EMCDDA and Europol (2015), *EMCDDA–Europol 2014 annual report on the implementation of Council Decision 2005/387/JHA* (https://www.emcdda.europa.eu/publications/implementation-reports/2014_en).

EMCDDA and Europol (2017), *Drugs and the Darknet – Perspectives for enforcement, research and policy*, EMCDDA–Europol Joint publications, Publications Office of the European Union, Luxembourg (https://www.emcdda.europa.eu/publications/joint-publications/drugs-andthe-darknet_en).

- EMCDDA and Europol (2019), *EU Drug Markets Report 2019*, Publications Office of the European Union, Luxembourg (https://www.emcdda.europa.eu/publications/joint-publications/eu-drug-markets-report-2019_en).
- Expertise Collective (2012), *Medicaments Psychotropes – Consommation et Pharmacodependances*, French National Institute of Health and Medical Research, Paris.
- Ford, L. T. and Berg, J. D. (2018), 'Analytical evidence to show letters impregnated with novel psychoactive substances are a means of getting drugs to inmates within the UK prison service', *Annals of Clinical Biochemistry*, Vol. 55, No 6, pp. 673–678 (https://doi.org/10.1177/0004563218767462).
- Fracasso, C., Confalonieri, S., Garattini, S. and Caccia, S. (1991), 'Single and multiple dose pharmacokinetics of etizolam in healthy subjects', *European Journal of Clinical Pharmacology*, Vol. 40, No 2, pp. 181–185 (https://doi.org/10.1007/BF00280074).
- Fraser, A. D., Isner, A. F. and Bryan, W. (1993), 'Urinary screening for adinazolam and its major metabolites by the Emit® d.a.u:™ and FPIA benzodiazepine assays with confirmation by HPLC', *Journal of Analytical Toxicology*, Vol. 17, pp. 427–431 (https://doi.org/10.1093/jat/17.7.427).
- Fukami, G., Hashimoto, T., Shirayama, Y., Hasegawa, T., Watanabe, H., Fujisaki, M., Hashimoto, K. and Iyo, M. (2010), 'Effects of etizolam and ethyl loflazepate on the P300 event-related potential in healthy subjects', *Annals of General Psychiatry*, Vol. 9, No 37 (https://doi.org/10.1186/1744-859X-9-37).
- Fukasawa, T., Yasui-Furukori, N., Suzuki, A., Inoue, Y., Tateishi, T. and Otani, K. (2005), 'Pharmacokinetics and pharmacodynamics of etizolam are influenced by polymorphic CYP2C19 activity', *European Journal of Clinical Pharmacology*, Vol. 61, No 11, pp. 791–795 (https://doi.org/10.1007/s00228-005-0032-8).
- Fukasawa, T., Suzuki, A. and Otani, K. (2007), 'Effects of genetic polymorphism of cytochrome P450 enzymes on the pharmacokinetics of benzodiazepines', *Journal of Clinical Pharmacy and Therapeutics*, Vol. 32, No 4, pp. 333–341 (https://doi.org/10.1111/j.1365-2710.2007.00829.x).
- Fukuda, T. and Tsumagari, T. (1983), 'Effects of psychotropic drugs on the rage responses induced by electrical stimulation of the medial hypothalamus in cats', *Japanese Journal of Pharmacology*, Vol. 33, No 4, pp. 885–890 (https://doi.org/10.1254/jjp.33.885).
- Garattini, S., Mussini, E., Marcucci, F. and Guaitani, A. (1973), 'Metabolic studies on benzodiazepines in various animal species', in Garattini, S., Mussini, E. and Randall, L.O. (eds), *The Benzodiazepines*, Raven Press, New York, pp. 75–97.
- Gavish, M., Bachman, I., Shoukrun, R., Katz, Y., Veenman, L., Weisinger, G and Weizman, A. (1999), 'Enigma of the peripheral benzodiazepine receptor', *Pharmacological Reviews*, Vol. 51, pp. 629–650.
- Goldstein, J. A. (2001), 'Clinical relevance of genetic polymorphisms in the human CYP2C subfamily', *British Journal of Clinical Pharmacology*, Vol. 52, No 4, pp. 349–355 (https://doi.org/10.1046/j.0306-5251.2001.01499.x).
- Gonzalez López, F, Mariño, E. L. and Dominguez-Gil, A. (1986), 'Pharmacokinetics of tiadipone: a new anxiolytic', *International Journal of Clinical Pharmacology, Therapy, and Toxicology*, Vol. 24, No 9, pp. 482–484.
- Gorski, J. C., Jones, D. R., Hamman, M. A., Wrighton, S. A. and Hall, S. D. (1999), 'Biotransformation of alprazolam by members of the human cytochrome P4503A subfamily', *Xenobiotica*, Vol. 29, pp. 931–944 (https://doi.org/10.1080/004982599238173).
- Greenblatt, D. (1981), 'Clinical pharmacokinetics of oxazepam and lorazepam', *Clinical Pharmacokinetics*, Vol. 6, pp. 89–105 (https://doi.org/10.2165/00003088-198106020-00001).
- Greenblatt, D. J., Divoll, M., Abernethy, D. R., Ochs, H. R. and Shader, R. I. (1983), 'Clinical pharmacokinetics of the newer benzodiazepines', *Clinical Pharmacokinetics*, Vol. 8, No 3, pp. 233–252 (https://doi.org/10.2165/00003088-198308030-00003).
- Griffiths, R. R. and Weerts, E. M. (1997), 'Benzodiazepine self-administration in humans and laboratory animals – implications for problems of long-term use and abuse', *Psychopharmacology*, Vol. 134, No 1, pp. 1–37 (https://doi.org/10.1007/s002130050422).
- Gupta, S. and Garg, B. (2014), 'A case of etizolam dependence', *Indian Journal of Pharmacology, Vol.* 46(6), pp. 655-656 (https://doi:10.4103/0253-7613.144943).
- Hester, J. B. and Von Voigtlander, P. (1979), '6-Aryl-4H-s-triazolo[4,3-a][1,4]benzodiazepines. Influence of 1-substitution on pharmacological activity', *Journal of Medicinal Chemistry*, Vol. 22, pp. 1390–1398 (https://doi.org/10.1021/jm00197a021).
- Hester Jr, J. B. (1976), '6-Phenyl-4H-s-triazolo[4.3-a][1.4] benzodiazepines', US patent No 3987052A. (https://patents.google.com/patent/US3987052A/en).
- Hester Jr, J. B., Rudzik, A. D. and Kamdar, B. V. (1971), '6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepines which have central nervous system depressant activity', *Journal of Medicinal Chemistry*, Vol. 14, No 11, pp. 1078–1081 (https://doi.org/10.1021/jm00293a015).
- Høiseth, G., Tuv, S. S. and Karinen, R. (2016), 'Blood concentrations of new designer benzodiazepines in forensic cases', *Forensic Science International*, Vol. 268, pp. 35–38 (https://doi.org/10.1016/j.forsciint.2016.09.006).
- Huppertz, L., Bisel, P., Westphal, F., Franz, F., Auwärter, V. and Moosmann, B. (2015), 'Characterization of the four designer benzodiazepines clonazolam, deschloroetizolam, flubromazolam, and meclonazepam, and identification of their in vitro metabolites', *Forensic Toxicology*, Vol. 33, pp. 388–395 (https://doi.org/10.1007/s11419-015-0277-6).
- Huppertz, L. M., Moosmann, B. and Auwärter, V. (2018), 'Flubromazolam – basic pharmacokinetic evaluation of a highly potent designer benzodiazepine', *Drug Testing and Analysis*, Vol. 10, No 1, pp. 206–211 (https://doi.org/10.1002/dta.2203).

Hyland, R., Osborne, T., Payne, A., Kempshall, S., Logan, Y. R., Ezzeddine, K. and Jones, B. (2009), 'In vitro and in vivo glucuronidation of midazolam in humans', *British Journal of Clinical Pharmacology*, Vol. 67, pp. 445–454 (https://doi.org/10.1111/j.1365-2125.2009.03386.x).

Iqbal, M. M., Sobhan, T. and Ryals, T. (2002), 'Effects of commonly used benzodiazepines on the fetus, the neonate, and the nursing infant', *Psychiatric Services*, Vol. 53, No 1, pp. 39–49 (https://doi.org/10.1176/appi.ps.53.1.39).

Johnson, D. N. and Funderburk, W. H. (1978), 'AHR-3219, a new antianxiety agent', *Progress in Neuropsychopharmacology*, Vol. 2, No 4, pp. 443–448 (https://doi.org/10.1016/0364-7722(78)90102-9).

Jones, J. D., Mogali, S. and Comer, S. D. (2012), 'Polydrug abuse: a review of opioid and benzodiazepine combination use', *Drug and Alcohol Dependence*, Vol. 125, No 1–2, pp. 8–18 (https://doi.org/10.1016/j.drugalcdep.2012.07.004).

Kilicarslan, T., Haining, R. L., Rettie, A. E., Busto, U., Tyndale, R. F. and Sellers, E. M. (2001), 'Flunitrazepam metabolism by cytochrome P450s 2C19 and 3A4', *Drug Metabolism and Disposition*, Vol. 29, pp. 460–465.

Kintz, P., Richeval, C., Jamey, C., Ameline, A., Allorge, D., Gaulier, J. M. and Raul, J. S. (2017), 'Detection of the designer benzodiazepine metizolam in urine and preliminary data on its metabolism', *Drug Testing and Analysis*, Vol. 9, No 7, pp. 1026–1033 (https://doi.org/10.1002/dta.2099).

Kitagawa, H., Esumi, Y., Kurosawa, S., Sekine, S. and Yokoshima, T. (1979), 'Metabolism of 8-chloro-6-(o-chlorophenyl)-1-methyl-4H-s-triazolo [4,3-a] [1,4] benzodiazepine, triazolam, a new central depressant. I. Absorption, distribution and excretion in rats, dogs and monkeys', *Xenobiotica*, Vol. 9, pp. 415–428 (https://doi.org/10.3109/00498257909038746).

Kleemann, A., Engel, J., Kutscher, B. and Reichert, D. (2009), *Pharmaceutical Substances – Syntheses, patents and applications of the most relevant APIs*, 5th edition, Thieme, Stuttgart.

Koch, K., Auwärter, V., Hermanns-Clausen, M., Wilde, M. and Neukamm, M. A. (2018), 'Mixed intoxication by the synthetic opioid U-47700 and the benzodiazepine flubromazepam with lethal outcome: pharmacokinetic data', *Drug Testing and Analysis*, Vol. 10, pp. 1336–1341 (https://doi.org/10.1002/dta.2391).

Kopanitsa, M. V., Zhuk, O. V., Zinkovsky, V. G. and Krishtal, O. A. (2001), 'Modulation of $GABA_A$ receptor mediated currents by phenazepam and its metabolites', *Naunyn Schmiedeberg's Archives of Pharmacology*, Vol. 364, No 1, pp. 1–8 (https://doi.org/10.1007/s002100100403).

Kopanitsa, M. V., Yakubovska, L. M., Rudenko, O. P. and Krishtal, O. A. (2002), 'Modulation of GABA(A) receptor-mediated currents by benzophenone derivatives in isolated rat Purkinje neurones', *Neuropharmacology*, Vol. 43, No 4, pp. 764–777 (https://doi.org/10.1016/s0028-3908(02)00120-x).

Kriikku, P., Rasanen, I., Ojanperä, I., Thelander, G., Kronstrand, R. and Vikingsson, S. (2020), 'Femoral blood concentrations of flualprazolam in 33 postmortem cases', *Forensic Science International*, Vol. 307, No 110101 (https://doi.org/10.1016/j.forsciint.2019.110101).

Lahti, R. A., Sethy, V. H., Barsuhn, C. and Hester, J. B. (1983), 'Pharmacological profile of the antidepressant adinazolam, a triazolobenzodiazepine', *Neuropharmacology*, Vol. 22, pp. 1277–1282 (https://doi.org/10.1016/0028-3908(83)90200-9).

Laing, M. K., Ti, L., Marmel, A., Tobias, S., Shapiro, A. M., Laing, R., Lysyshyn, M. and Socías, M. E. (2021), 'An outbreak of novel psychoactive substance benzodiazepines in the unregulated drug supply: preliminary results from a community drug checking program using point-of-care and confirmatory methods', *The International Journal on Drug Policy*, Vol. 103169 (https://doi.org/10.1016/j.drugpo.2021.103169).

Leonelli, E., Yague, J. G., Ballabio, M., Azcoitia, I., Magnaghi, V., Schumacher, M., Garcia-Segura, L. M. and Melcangi, R. C. (2005), 'Ro5-4864, a synthetic ligand of peripheral benzodiazepine receptor, reduces aging-associated myelin degeneration in the sciatic nerve of male rats', *Mechanisms of Ageing and Development*, Vol. 126, No 11, pp. 1159–1163 (https://doi.org/10.1016/j.mad.2005.06.001).

Lim, W. J., Yap, A. T., Mangudi, M., Koh, H. B., Tang, A. S. and Chan, K. B. (2017), 'Detection of phenazepam in illicitly manufactured Erimin 5 tablets', *Drug Testing and Analysis*, Vol. 9, No 2, pp. 293–305 (https://doi.org/10.1002/dta.1981).

Łukasik-Głębocka, M., Sommerfeld, K., Tężyk, A., Zielińska-Psuja, B., Panieński, P. and Żaba, C. (2016), 'Flubromazolam – a new life-threatening designer benzodiazepine', *Clinical Toxicology*, Vol. 54, No 1, pp. 66–68 (https://doi.org/10.3109/15563650.2015.1112907).

Manchester, K. R., Maskell, P. D. and Waters, L. (2018), 'Experimental versus theoretical log $D_{7.4}$, pKa and plasma protein binding values for benzodiazepines appearing as new psychoactive substances', *Drug Testing and Analysis*, Vol. 10, pp. 1258–1269 (https://doi.org/10.1002/dta.2387).

Masica, A. L., Mayo, G. and Wilkinson, G. R. (2004), 'In vivo comparisons of constitutive cytochrome P450 3A activity assessed by alprazolam, triazolam, and midazolam', *Clinical Pharmacology and Therapeutics*, Vol. 76, pp. 341–349 (https://doi.org/10.1016/j.clpt.2004.07.003).

Maskell, P. D., De Paoli, G., Nitin Seetohul, L. and Pounder, D. J. (2012), 'Phenazepam: The drug that came in from the cold', *Journal of Forensic and Legal Medicine*, Vol. 19, pp. 122–125 (https://doi.org/10.1016/j.jflm.2011.12.014).

Mattila, M. A. K. and Larni, H. M. (1980), 'Flunitrazepam: a review of its pharmacological properties and therapeutic use', *Drugs*, Vol. 20, pp. 353–374 (https://doi.org/10.2165/00003495-198020050-00002).

Mei, V., Concheiro, M., Pardi, J. and Cooper, G. (2019), 'Validation of an LC-MS/MS method for the quantification of 13 designer benzodiazepines in blood', *Journal of Analytical Toxicology*, Vol. 43, No 9, pp. 688–695 (https://doi.org/10.1093/jat/bkz063).

- Melo, P., Bastos, M. L. and Teixeira, H. M. (2012), 'Benzodiazepine stability in postmortem samples stored at different temperatures', *Journal of Analytical Toxicology*, Vol. 36, No 1, pp. 52–60 (https://doi.org/10.1093/jat/bkr008).
- Meyer, M. R., Bergstrand, M. P., Helander, A. and Beck, O. (2016), 'Identification of main human urinary metabolites of the designer nitrobenzodiazepines clonazolam, meclonazepam, and nifoxipam by nano-liquid chromatography-high-resolution mass spectrometry for drug testing purposes', *Analytical and Bioanalytical Chemistry*, Vol. 408, No 13, pp. 3571–3591 (https://doi.org/10.1007/s00216-016-9439-6).
- Mills, C., Makwana, M., Wallace, A., Benn, S., Schmidt, H., Tegeder, I., Costigan, M., Brown Jr, R. H, Raivich, G. and Woolf, C. J. (2008), 'Ro5-4864 promotes neonatal motor neuron survival and nerve regeneration in adult rats', *The European Journal of Neuroscience*, Vol. 27, No 4, pp. 937–946 (https://doi.org/10.1111/j.1460-9568.2008.06065.x).
- Mizuno, K., Katoh, M., Okumura, H., Nakagawa, N., Negishi, T., Hashizume, T., Nakajima, M. and Yokoi, T. (2009), 'Metabolic activation of benzodiazepines by CYP3A4', *Drug Metabolism and Disposition*, Vol. 37, No 2, pp. 345–351 (https://doi.org/10.1124/dmd.108.024521).
- Mohsin, N. and Qadir, M. (2015), 'Recent structure–activity relationship studies of 1,4-benzodiazepines', *Peertechz Journal of Medicinal Chemistry and Research*, Vol. 1, No 1, pp. 008–0012 (https://doi.org/10.17352/ojc.000002).
- Molodavkin, G. M., Burlakova, E. B., Cherniavskaia, L. I., Voronina, T. A., Khorseva, N. I. and Seredenin, S. B. (1996), 'Farmakologicheskiĭ analiz aktivnosti fenazepama i flunitrazepama, vvodimykh v sverkhnizkikh dozakh' ['Pharmacological analysis of phenazepam and flunitrazepam activity when administered in extremely low doses'], *Bulletin of Experimental Biology and Medicine*, Vol. 121, No 2, pp. 164–166.
- Molodavkin, G. M., Voronina, T. A., Chernyavskaya, L. I., Burlakova, E. B., Khorseva, N. I. and Seredenin, S. B. (2003), 'Pharmacological activity of phenazepam and flunitrazepam in ultralow doses', *Bulletin of Experimental Biology and Medicine*, Vol. 135 (Suppl. 7), pp. 39–41 (https://doi.org/10.1023/a:1024710225445).
- Moody, D. (2004), 'Drug interactions with benzodiazepines', in Raymon, L. P. and Mozayani, A. (eds), *Handbook of Drug Interactions – A clinical and forensic guide*, Humana Press, Totowa, NJ, pp. 3–88.
- Moosmann, B. and Auwärter, V. (2018), 'Designer benzodiazepines: another class of new psychoactive substances', in Maurer, H. and Brandt, S. (eds), *New Psychoactive Substances – Handbook of experimental pharmacology*, Vol. 252, Springer, Cham, pp. 383–410 (https://doi.org/10.1007/164_2018_154).
- Moosmann, B., Hutter, M., Huppertz, L., Ferlaino, S., Redlingshöfer, L. and Auwärter, V. (2013a), 'Characterization of the designer benzodiazepine pyrazolam and its detectability in human serum and urine', *Forensic Toxicology*, Vol. 31, pp. 263–271 (https://doi.org/10.1007/s11419-013-0187-4).
- Moosmann, B., Huppertz, L. M., Hutter, M., Buchwald, A., Ferlaino, S. and Auwärter, V. (2013b), 'Detection and identification of the designer benzodiazepine flubromazepam and preliminary data on its metabolism and pharmacokinetics', *Journal of Mass Spectrometry*, Vol. 48, pp. 1150–1159 (https://doi.org/10.1002/jms.3279).
- Moosmann, B., Bisel, P. and Auwärter, V. (2014), 'Characterization of the designer benzodiazepine diclazepam and preliminary data on its metabolism and pharmacokinetics', *Drug Testing and Analysis*, Vol. 6, pp. 757–763 (https://doi.org/10.1002/dta.1628).
- Moosmann, B., Bisel, P., Franz, F., Huppertz, L. M. and Auwärter V. (2016), 'Characterization and *in vitro* phase I microsomal metabolism of designer benzodiazepines – an update comprising adinazolam, cloniprazepam, fonazepam, 3-hydroxyphenazepam, metizolam and nitrazolam', *Journal of Mass Spectrometry*, Vol. 51, No 11, pp. 1080–1089 (https://doi.org/10.1002/jms.3840).
- Moosmann, B., Bisel, P., Westphal, F., Wilde, M., Kempf, J., Angerer, V. and Auwärter, V. (2019), 'Characterization and *in vitro* phase I microsomal metabolism of designer benzodiazepines: an update comprising flunitrazolam, norflurazepam, and 4'-chlorodiazepam (Ro5-4864)', *Drug Testing and Analysis*, Vol. 11, No 3, pp. 541–549 (https://doi.org/10.1002/dta.2561).
- Mortelé, O., Vervliet, P., Gys, C., Degreef, M., Cuykx, M., Maudens, K., Covaci, A., van Nuijs, L. N. and Lai, F. Y. (2018), '*In vitro* phase I and phase II metabolism of the new designer benzodiazepine cloniprazepam using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry', *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 153, pp. 158–167 (https://doi.org/10.1016/j.jpba.2018.02.032).
- Nakamae, T., Shinozuka, T., Sasaki, C., Ogamo, A., Murakami-Hashimoto, C., Irie, W., Terada, M., Nakamura, S., Furukawa, M. and Kurihara, K. (2008), 'Case report: etizolam and its major metabolites in two unnatural death cases', *Forensic Science International*, Vol. 182, pp. e1–e6 (https://doi.org/10.1016/j.forsciint.2008.08.012).
- National Records of Scotland (2020), *Drug-related Deaths in Scotland in 2019* (https://www.nrscotland.gov.uk/files//statistics/drug-related-deaths/2019/drug-related-deaths-19-pub.pdf).
- Nielsen, S. and McAuley, A. (2020), 'Etizolam: a rapid review on pharmacology, non-medical use and harms', *Drug and Alcohol Review*, Vol. 39, No 4, pp. 330–336 (https://doi.org/10.1111/dar.13052).
- Nishii, S., Hori, H., Kishimoto, T. and Nakamura, J. (2014), 'A successful case of dose reduction in etizolam dependence using fine granules: a case report', *International Medical Case Reports Journal*, Vol. 7, pp. 121–122 (https://doi.org/10.2147/IMCRJ.S67103).
- Noble, C., Mardal, M., Bjerre Holm, N., Stybe Johansen, S. and Linnet, K. (2017), '*In vitro* studies on flubromazolam metabolism and detection of its metabolites in authentic forensic samples', *Drug Testing and Analysis*, Vol. 9, pp. 1182–1191 (https://doi.org/10.1002/dta.2146).

Olivera, M., Martínez, C., Gervasini, G., Carrillo, J. A., Ramos, S., Benítez, J., García-Martin, E. and Agundez, J. A. G. (2007), 'Effect of common NAT2 variant alleles in the acetylation of the major clonazepam metabolite, 7-aminoclonazepam', *Drug Metabolism Letters*, Vol. 1, No 1, pp. 3–5 (https://doi.org/10.2174/18723120 7779814283).

Olsen, R. W. and Sieghart W. (2008), 'International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update', *Pharmacological Reviews*, Vol. 60, No 3, pp. 243–260 (https://doi.org/10.1124/ pr.108.00505).

Panlilio, L. V., Thorndike, E. B. and Schindler, C. W. (2005), 'Lorazepam reinstates punishment-suppressed remifentanil self-administration in rats', *Psychopharmacology*, Vol. 179, No 2, pp. 374–382 (https://doi.org/10.1007/s00213-004-2040-2).

Papsun, D. M., Krotulski, A. J., Homan, J., Temporal, K. D. H. and Logan, B. K. (2021), 'Flualprazolam blood concentrations in 197 forensic investigation cases', *Journal of Analytical Toxicology*, Vol. 45, No 3, pp. 226–232 (https://doi.org/10.1093/jat/ bkaa070).

Pariante, F., Caddeo, S. and Ecari, U. (1989), 'Etizolam in the treatment of generalized anxiety disorder associated with depressive symptoms', *Current Medical Research and Opinion*, Vol. 11, No 9, pp. 543–549 (https://doi. org/10.1185/03007998909112670).

Parmeggiani, A., Posar, A., Sangiorgi, S. and Giovanardi-Rossi, P. (2004), 'Unusual side-effects due to clobazam: a case report with genetic study of CYP2C19', *Brain and Development*, Vol. 26, No 1, pp. 63–66 (https://doi.org/10.1016/s0387-7604(03)00074-3).

Pellow, S. and File, S. E. (1986), 'Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat', *Pharmacology, Biochemistry and Behavior*, Vol. 24, No 3, pp. 525–529 (https://doi. org/10.1016/0091-3057(86)90552-6).

Penninga, E. I., Graudal, N., Ladekarl, M. B. and Jürgens, G. (2016), 'Adverse events associated with flumazenil treatment for the management of suspected benzodiazepine intoxication – a systematic review with meta-analyses of randomised trials', *Basic and Clinical Pharmacology and Toxicology*, Vol. 118, No 1, pp. 37–44 (https://doi.org/10.1111/bcpt.12434).

Penninkilampi, R. and Eslick, G. D. (2018), 'A systematic review and meta-analysis of the risk of dementia associated with benzodiazepine use, after controlling for protopathic bias', *CNS Drugs*, Vol. 32, No 6, pp. 485–497 (https://doi.org/10.1007/ s40263-018-0535-3).

Peters Jr, R. J., Meshack. A. F., Kelder, S. H., Webb, P., Smith, D. and Garner, K. (2007), 'Alprazolam (Xanax) use among southern youth: beliefs and social norms concerning dangerous rides on "handlebars"', *Journal of Drug Education*, Vol. 37, No 4, pp. 417–428 (https://doi.org/10.2190/DE.37.4.e).

Pettersson Bergstrand, M., Helander, A. and Beck, O. (2016), 'Development and application of a multi-component LC-MS/MS method for determination of designer benzodiazepines in urine', *Journal of Chromatography B Analytical Technologies in the Biomedical and Life Sciences*, Vol. 1035, pp. 104–110 (https:// doi.org/10.1016/j.jchromb.2016.08.047).

Pettersson Bergstrand, M., Helander, A., Hansson, T. and Beck, O. (2017), 'Detectability of designer benzodiazepines in CEDIA, EMIT II Plus, HEIA, and KIMS II immunochemical screening assays', *Drug Testing and Analysis*, Vol. 9, No 4, pp. 640–645 (https://doi.org/10.1002/dta.2003).

Pettersson Bergstrand, M., Meyer, M. R., Beck, O. and Helander, A. (2018), 'Human urinary metabolic patterns of the designer benzodiazepines flubromazolam and pyrazolam studied by liquid chromatography-high resolution mass spectrometry', *Drug Testing and Analysis*, Vol. 10, No 3, pp. 496–506 (https://doi. org/10.1002/dta.2243).

Pope, J. D., Choy, K. W., Drummer, O. H. and Schneider, H. G. (2018), 'Novel benzodiazepines (clonazolam and flubromazolam) identified in candy-like pills', *The Journal of Applied Laboratory Medicine*, Vol. 3, No 1, pp. 48–55 (https://doi.org/10.1373/ jalm.2017.025387).

Public Health England (2020), *Drug Alert – Evidence of harm from fake benzodiazepine medicines containing new (designer) benzodiazepines: England, United Kingdom, 24 July 2020* (https://www.cas.mhra.gov.uk/ViewandAcknowledgment/ ViewAlert.aspx?AlertID=103075).

Qian, Z., Liu, C., Huang, J., Deng, Q. and Hua, Z. (2020), 'Identification of the designer benzodiazepine 8-chloro-6-(2-fluorophenyl)-1-methyl-4H-[1,2,4]triazolo[4,3-a][1,4] benzodiazepine (flualprazolam) in an anesthesia robbery case', *Forensic Toxicology*, Vol. 38, pp. 269–276 (https://doi. org/10.1007/s11419-019-00501-1).

Robertson, M. D. and Drummer, O. H. (1998), 'Stability of nitrobenzodiazepines in postmortem blood', *Journal of Forensic Sciences*, Vol. 43, No 1, pp. 5–8 (https://doi.org/10.1520/ JFS16081J).

Rupprecht, R., Rammes, G., Eser, D., Baghai, T. C., Schüle, C., Nothdurfter, C., et al. (2009), 'Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects', *Science*, Vol. 325, No 5939, pp. 490–493 (https://doi. org/10.1126/science.1175055).

Sanna, E., Pau, D., Tuveri, F., Massa, F., Maciocco, E., Acquas, C., Floris, C., Fontana, S. N., Maira, G. and Biggio, G. (1999), 'Molecular and neurochemical evaluation of the effects of etizolam on $GABA_A$ receptors under normal and stress conditions', *Arzneimittelforschung*, Vol. 49, pp. 88–95 (https:// doi.org/10.1055/s-0031-1300366).

Savoldi, F., Somenzini, G. and Ecari, U. (1990), 'Etizolam versus placebo in the treatment of panic disorder with agoraphobia: a double-blind study', *Current Medical Research and Opinion*, Vol. 12, No 3, pp. 185–190 (https://doi. org/10.1185/03007999009111500).

Schukin S. I., Zinkovsky, V. G. and Zhuk, O. V. (2011), 'Elimination kinetics of the novel prodrug cinazepam possessing psychotropic activityin mice', *Pharmacological Reports*, Vol. 63,

No 5, pp. 1093–1100 (https://doi.org/10.1016/S1734-1140(11)70628-4).

Schulz, M., Iwersen-Bergmann, S., Andresen, H. and Schmoldt, A. (2012), 'Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics', *Critical Care*, Vol. 16, No R136 (https://doi.org/10.1186/cc11441).

Schwartz, M. A., Koechlin, B. A., Postma, E., Palmer, S. and Krol, G. (1965), 'Metabolism of diazepam in rat, dog, and man', *The Journal of Pharmacology and Experimental Therapeutics*, Vol. 149, pp. 423–435.

Sigel, E., Schaerer, M. T., Buhr, A. and Baur, R. (1998), 'The benzodiazepine binding pocket of recombinant α1β2γ2 γ-aminobutyric acid$_A$ receptors: Relative orientation of ligands and amino acid side chains', *Molecular Pharmacology*, Vol. 54, No 6, pp. 1097–1105 (https://doi.org/10.1124/mol.54.6.1097).

Skopp, G. (2004), 'Preanalytic aspects in postmortem toxicology', *Forensic Science International*, Vol. 142, No 2–3, pp. 75–100 (https://doi.org/10.1016/j.forsciint.2004.02.012).

Skopp, G., Pötsch, L., König, I. and Mattern, R. (1998), 'A preliminary study on the stability of benzodiazepines in blood and plasma stored at 4 °C', *International Journal of Legal Medicine*, Vol. 111, No 1, pp. 1–5 (https://doi.org/10.1007/s004140050100).

Starcevic, V. (2014), 'The reappraisal of benzodiazepines in the treatment of anxiety and related disorders', *Expert Review in Neurotherapeutics*, Vol. 14, No 11, pp. 1275–1286 (https://doi.org/10.1586/14737175.2014.963057).

Sternbach, L. H. (1971), '1,4-Benzodiazepines. Chemistry and some aspects of the structure–activity relationship', *Angewandte Chemie*, Vol. 10 (international edition in English), pp. 34–43 (https://doi.org/10.1002/anie.197100341).

Sternbach, L. H. (1979), 'The benzodiazepine story', *Journal of Medicinal Chemistry*, Vol. 22, pp. 1–7 (https://doi.org/10.1021/jm00187a001).

Sternbach, L. H., Fryer, R. I., Metlesics, W., Reeder, E., Sach, G., Saucy, G. and Stempel, A. (1962), 'Quinazolines and 1,4-benzodiazepines. VI.1a halo-, methyl-, and methoxy-substituted 1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-ones1b,c', *The Journal of Organic Chemistry*, Vol. 27, pp. 3788–3796 (https://doi.org/10.1021/jo01058a010).

Sternbach, L. H., Randall, L. O., Banziger, R. and Lehr, H. (1968), 'Structure–activity relationships in the 1,4-benzodiazepine series', in Burger, A. (ed.), *Drugs Affecting the Central Nervous System, Vol 2,* Edward Arnold, London.

Stone, B. T., Correa, K. A., Brown, T. L., Spurgin, A. L., Stikic, M., Johnson, R. R. and Berka, C. (2015), 'Behavioral and neurophysiological signatures of benzodiazepine-related driving impairments', *Frontiers in Psychology*, Vol. 6, p. 1799 (https://doi.org/10.3389/fpsyg.2015.01799).

Tahara, T., Araki, K., Shiroki, M., Matsuo, H. and Munakata, T. (1978), 'Syntheses and structure–activity relationships of 6-aryl-4H-s-triazolo[3,4-c]thieno[2,3-e] [1,4]diazepines', *Arzneimittelforschung*, Vol. 28, No 7, pp. 1153–1158 (https://doi.org/10.1002/chin.197844214).

Tan, K. R., Rudolph, U. and Lüscher, C. (2011), 'Hooked on benzodiazepines: GABA$_A$ receptor subtypes and addiction', *Trends in Neurosciences*, Vol. 34, pp. 188–197 (https://doi.org/10.1016/j.tins.2011.01.004).

Tsumagari, T., Nakajima, A., Fukuda, T., Shuto, S., Kenjo, T., Morimoto, Y. and Takigawa, Y. (1978), 'Pharmacological properties of 6-(o-chlorophenyl)-8-ethyl-1-methyl-4H-s-triazolo[3,4-c]thieno[2,3-e] [1,4]diazepine (Y-7131), a new anti-anxiety drug', *Arzneimittelforschung*, Vol. 28, No 7, pp. 1158–1164.

UNODC (United Nations Office on Drugs and Crime) (1997), *Recommended methods for the detection and assay of barbiturates and benzodiazepines in biological specimens – Manual for use by National Laboratories*, UN document ID No ST/NAR/28, UNODC, New York.

UNODC (2019), *Current NPS Threats – Volume 1: March 2019* (https://www.unodc.org/pdf/opioids-crisis/Current_NPS_Threats_-_Volume_I.pdf).

UNODC (2020), *International Control of DOC, AB-FUBINACA, 5F-AMB-PINACA, 5F-MDMB-PICA, 4FMDMB-BINACA, 4-chloromethcathinone, N-ethylhexedrone, α-pyrrolidinohexanophenone, flualprazolam and etizolam will enter into force on 3 November 2020* (https://www.unodc.org/LSS/Announcement/Details/64167b6d-968d-4d0c-bc65-5deb99683191).

Van der Bijl, P. and Roelofse, J. A. (1991), 'Disinhibitory reactions to benzodiazepines: a review', *Journal of Oral and Maxillofacial Surgery*, Vol. 49, No 5, pp. 519–523 (https://doi.org/10.1016/0278-2391(91)90180-t).

Vikingsson, S., Wohlfarth, A., Andersson, M., Gréen, H., Roman, M., Josefsson, M., Kugelberg, F. C. and Kronstrand, R. (2017), 'Identifying metabolites of meclonazepam by high-resolution mass spectrometry using human liver microsomes, hepatocytes, a mouse model, and authentic urine samples', *The AAPS Journal*, Vol. 19, pp. 736–742 (https://doi.org/10.1208/s12248-016-0040-x).

Vogt, S., Kempf, J., Buttler, J., Auwärter, V. and Weinmann, W. (2013), 'Desalkylflurazepam found in patients' samples after high-dose midazolam treatment', *Drug Testing and Analysis*, Vol. 5, No 9–10, pp. 745–747 (https://doi.org/10.1002/dta.1484).

Wagmann, L., Manier, S. K., Bambauer, T. P., Felske, C., Eckstein, N., Flockerzi, V. and Meyer, M. R. (2020), 'Toxicokinetics and analytical toxicology of flualprazolam: metabolic fate, isozyme mapping, human plasma concentration, and main urinary excretion products', *Journal of Analytical Toxicology*, Vol. 44, No 6, pp. 549–558 (https://doi.org/10.1093/jat/bkaa019).

Walker, B. M. and Ettenberg, A. (2005), 'Intra-ventral tegmental area heroin-induced place preferences in rats are potentiated by peripherally administered alprazolam', *Pharmacology, Biochemistry, and Behavior*, Vol. 82, No 3, pp. 470–477 (https://doi.org/10.1016/j.pbb.2005.10.002).

Waters, L., Manchester, K. R., Maskell, P. D., Haider, S. and Haegeman, C. (2018), 'The use of a quantitative structure–activity relationship (QSAR) model to predict GABA-A receptor binding of newly emerging benzodiazepines', *Science and Justice*, Vol. 58, No 3, pp. 219–225 (https://doi.org/10.1016/j.scijus.2017.12.004).

Wedinos (Welsh Emerging Drugs and Identification of Novel Substances Project) (2020), project website (https://www.wedinos.org).

WHO (World Health Organization) (1989), 'WHO Expert Committee on Drug Dependence: twenty-sixth report', *WHO Technical Report Series*, No 787 (https://apps.who.int/iris/handle/10665/41542).

WHO (1991), 'WHO Expert Committee on Drug Dependence: twenty-seventh report', *WHO Technical Report Series*, No 808 (https://apps.who.int/iris/handle/10665/40616).

WHO (2016), 'WHO Expert Committee on Drug Dependence: thirty-seventh report', *WHO Technical Report Series*, No 998 (https://apps.who.int/iris/handle/10665/206452).

WHO (2018), 'WHO Expert Committee on Drug Dependence: thirty-ninth report', *WHO Technical Report Series*, No 1009 (https://apps.who.int/iris/handle/10665/260546).

WHO (2020a), 'WHO Expert Committee on Drug Dependence: forty-second report', *WHO Technical Report Series*, No 1026 (https://apps.who.int/iris/bitstream/handle/10665/331486/9789240001848-eng.pdf).

WHO (2020b), *Critical Review Report – Clonazolam*, Expert Committee on Drug Dependence, Forty-third Meeting, Geneva, 12–20 October 2020 (https://www.who.int/docs/default-source/controlled-substances/43rd-ecdd/final-clonazolam-a.pdf?sfvrsn=b8b10967_4).

WHO (2020c), *Critical Review Report – Diclazepam*, Expert Committee on Drug Dependence, Forty-third Meeting, Geneva, 12–20 October 2020 (https://www.who.int/docs/default-source/controlled-substances/43rd-ecdd/final-diclazepam-a.pdf?sfvrsn=dea906ed_4).

WHO (2020d), *Critical Review Report – Flubromazolam*, Expert Committee on Drug Dependence, Forty-third Meeting, Geneva, 12–20 October 2020 (https://www.who.int/docs/default-source/controlled-substances/43rd-ecdd/final-flubromazolam-a.pdf?sfvrsn=887bdf43_4).

Wohlfarth, A., Vikingsson, S., Roman, M., Andersson, M., Kugelberg, F. C., Green, H. and Kronstrand, R. (2017), 'Looking at flubromazolam metabolism from four different angles: metabolite profiling in human liver microsomes, human hepatocytes, mice and authentic human urine samples with liquid chromatography high-resolution mass spectrometry', *Forensic Science International*, Vol. 274, pp. 55–63 (https://doi.org/10.1016/j.forsciint.2016.10.021).

Woods, J. H., Katz, J. L. and Winger, G. (1987), 'Abuse liability of benzodiazepines', *Pharmacological Reviews*, Vol. 39, No 4, pp. 251–413. Erratum in *Pharmacological Reviews*, Vol. 40, No 1, p. 85.

Woods, J. H., Katz, J. L. and Winger, G. (1992), 'Benzodiazepines: use, abuse, and consequences', *Pharmacological Reviews*, Vol. 44, No 2, pp. 151–347.

Woolverton, W. L. and Nader, M. A. (1995), 'Effects of several benzodiazepines, alone and in combination with flumazenil, in rhesus monkeys trained to discriminate pentobarbital from saline', *Psychopharmacology*, Vol. 122, No 3, pp. 230–236 (https://doi.org/10.1007/BF02246544).

Xiang, P., Shen, B. H., Yan, H., Liu, W., Shen, M., Wu, H. J. and Zhuo, X. Y. (2018), 'Identification of new designer benzodiazepine diclazepam in drug facilitated sexual assault', *Fa Yi Xue Za Zhi*, Vol. 34, No 3, pp. 248–252 (https://doi.org/10.12116/j.issn.1004-5619.2018.03.006).

Xu, C., Li, C. Y. and Kong, A. N. (2005), 'Induction of phase I, II and III drug metabolism/transport by xenobiotics', *Archives of Pharmacal Research*, Vol. 28, No 3, pp. 249–268 (https://doi.org/10.1007/BF02977789).

Yakushiji, T., Fukuda, T., Oyama, Y. and Akaike, N. (1989), 'Effects of benzodiazepines and nonbenzodiazepine compounds on the GABA-induced response in frog isolated sensory neurones', *British Journal of Pharmacology*, Vol. 98, No 3, pp. 735–740 (https://doi.org/10.1111/j.1476-5381.1989.tb14600.x).

Zherdev, V. P., Caccia, S., Garattini, S. and Ekonomov, A. L. (1982), 'Species differences in phenazepam kinetics and metabolism', *European Journal of Drug Metabolism and Pharmacokinetics*, Vol. 7, No 3, pp. 191–196 (https://doi.org/10.1007/BF03189565).

Annex – Profiles of selected new benzodiazepines

Flualprazolam

Background information

Flualprazolam can be regarded as the 2'-fluoro derivative of alprazolam or an analogue of flubromazolam (exchange of the bromine atom by a chlorine atom), and was first mentioned in a patent filed in 1969 by The Upjohn Company under the name 8-chloro-1-methyl-6-(o-fluorophenyl)-4*H*-s-triazolo[4,3-*a*][1,4]-benzodiazepine. In this patent, the synthesis of various triazolo-1,4-benzodiazepines by reaction of 1,4-benzodiazepine-2-thione precursors with acetic acid hydrazide was described. Although the patent was finally granted in 1976 (Hester, 1976), flualprazolam has never been marketed by The Upjohn Company.

Its first appearance on the market for NPS dates back to 2017.

Physical and chemical description

Flualprazolam is a white powder in its pure form.

IUPAC name: 8-Chloro-6-(2-fluorophenyl)-1-methyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine.

Chemical Abstracts Service (CAS) Number: 28910-91-0.

Sum formula: $C_{17}H_{12}ClFN_4$.

Molecular mass: 326.7554 g/mol.

Monoisotopic mass: 326.0735 u; $[M+H]^+$: 327.0807 u.

Melting point: 203–204 °C (Hester, 1976).

Predicted log $D_{7.4}$ (using ACD/Labs Percepta Platform – PhysChem Module): 2.44 (http://www.chemspider.com/Chemical-Structure.8534493.html).

Pharmacology

Owing to the structural similarity with the triazolobenzodiazepines alprazolam and triazolam, it can be expected that the potency of flualprazolam is comparable (a dose equivalent to 10 mg of diazepam is about 0.5 mg for alprazolam and triazolam) and that it is rapidly absorbed after oral ingestion. Considering the pattern of halogen substitution, the elimination half-life of flualprazolam is likely to be in a range similar to that of triazolam (approximately 2 hours) or alprazolam (6–12 hours).

In the patent by Hester (Hester, 1976), the following ED_{50} values, in mg/kg, are given for flualprazolam for mice after intraperitoneal administration: 0.056 (chimney test: ability of mice to back up and out of a vertical glass cylinder within 30 seconds), 0.016 (dish test: mice remain in a Petri dish for more than 3 minutes), 0.028 (pedestal test: mice stay in a pedestal for more than 1 minute), 0.009 (nicotine antagonism test: protection from overstimulation and death of mice after injection of 2 mg/kg of nicotine salicylate prior to flualprazolam administration).

On TripSit (http://drugs.tripsit.me/flualprazolam, accessed October 2019), the following data are given, which probably originate from non-controlled self-experiments and have to be considered with caution: a common oral dose is given as 0.25–0.5 mg (light dose, 0.125–0.25 mg; strong dose, 0.5–1 mg; heavy dose, 1–2 mg). Onset of action is stated to occur 10–30 minutes after ingestion, duration of action is 6–14 hours and after-effects last 1–36 hours.

Toxicology

There are no data available on the acute or chronic toxicity of flualprazolam, but because of the structural similarities to well-characterised triazolobenzodiazepines such as triazolam, toxicity can be anticipated to be similarly low.

Mei et al. (2019) published a validated LC-MS/MS method for the detection and quantification of 13 new benzodiazepines, including flualprazolam, in blood samples.

Wagmann et al. (2020) published a study on the metabolism of flualprazolam. Seven flualprazolam metabolites were tentatively identified. Several CYP and UGT isozymes, amongst them CYP3A4 and UGT1A4, were shown to be involved in flualprazolam biotransformation reactions. Alpha-hydroxy flualprazolam glucuronide, 4-hydroxy flualprazolam glucuronide and the parent glucuronide were identified as the most abundant signals in urine, far more abundant than the parent compound flualprazolam.

Kriikku et al. (2020) reported the quantitative confirmation of flualprazolam in 33 post-mortem cases in Sweden and Finland. From this study, the median concentration of flualprazolam in blood was 18.0 ng/g (ranging from 3.0 to 68 ng/g); 13 cases listed flualprazolam as a cause of death.

Papsun et al. (2021) reported that flualprazolam was detected in 197 blood samples from medicolegal death investigations and human performance cases reported between August 2019 and February 2020 in the United States. A total of 171 cases were samples collected during medicolegal death (i.e. post-mortem) investigations and 22 were from human performance (i.e. DUID) investigations; four cases were submitted under unknown circumstances. In post-mortem cases with quantitative results (n = 167), the mean (± SD) flualprazolam concentration was 20 (± 63) ng/ml, the median concentration was 8.2 ng/ml, and the range of concentrations was 2.0–620 ng/ml. Four remaining post-mortem cases were reported to have tested positive for flualprazolam, however, flualprazolam has not been quantified in those cases (< 2.0 ng/ml). In drug-impaired driving cases (n = 22), the mean (± SD) flualprazolam concentration was 22 (± 18) ng/ml, the median concentration was 14 ng/ml and the range of concentrations was 4.4–68 ng/ml. Flualprazolam was commonly (83 % of the time) found in combination with opioids (e.g. fentanyl).

Dependence and abuse potential

There are no data available on the dependence and abuse potential of flualprazolam. However, it can be assumed that the abuse liability and the potential to produce dependence are similar to those of other chemically related triazolobenzodiazepines such as alprazolam or triazolam.

Epidemiology

There are no data on the use of flualprazolam in the general population.

In general, benzodiazepines play a major role in DUID offences, and flualprazolam has recently been reported in a few DUID cases, which mainly occurred in the United States (UNODC, 2019). A case in which flualprazolam was used in an anaesthesia robbery case in China in November 2017 has also been reported (Qian et al., 2020).

Flubromazolam

Background information

Flubromazolam can be regarded as the triazolo derivative of the new benzodiazepine flubromazepam; it was one of the first new benzodiazepines entering the market in 2014, and, from its chemical structure, it is closely related to flualprazolam and triazolam. It is covered by a patent held by The Upjohn Company and granted in 1972, but specific data on this compound were not included.

The first appearance of flubromazolam as a new benzodiazepine offered in online shops dates back to 2014. Flubromazolam has been detected in fake Xanax (alprazolam) tablets (EMCDDA, 2020). According to another report (Pope et al., 2018), candy-like pills found on an intoxicated patient participating in a methadone programme contained flubromazolam (0.18 mg) next to a much higher dose of clonazolam (16 mg). A urine sample of the patient was found to be positive for flubromazolam and its metabolites (Pope et al., 2018).

Andersson and Kjellgren (2017) recently evaluated discussions on a Swedish online forum (https://www.flashback.org/) regarding flubromazolam and concluded that this new benzodiazepine is generally described as very potent and long-lasting. It was rated as highly addictive and can lead to many, possibly severe, side effects such as memory loss and loss of control.

Physical and chemical description

Flubromazolam is a white powder in its pure form.

IUPAC name: 8-Bromo-6-(2-fluorophenyl)-1-methyl-4*H*-[1,2,4]triazolo-[4,3-*a*][1,4]benzodiazepine.

CAS Number: 612526-40-6.

Sum formula: $C_{17}H_{12}BrFN_4$.

Molecular mass: 371.2064 g/mol.

Monoisotopic mass: 370.0229 u; $[M+H]^+$: 371.0302 u

Melting point: 213–214 °C (patent US 20100130481, compound [0075] (Cook et al., 2010)); 205.3 °C (swgdrug monograph of flubromazolam, revision 13 January 2017 (http://www.swgdrug.org/Monographs/Flubromazolam.pdf)).

Predicted log $D_{7.4}$ (using ACD/Labs Percepta Platform – PhysChem Module): 2.50 (http://www.chemspider.com/Chemical-Structure.10684757.html).

Experimentally determined log $D_{7.4}$: 2.40 (Manchester et al., 2018).

Pharmacology

Owing to the structural similarity to the triazolobenzodiazepines alprazolam and triazolam, flubromazolam can be expected to be similarly potent (a dose equivalent to 10 mg of diazepam is about 0.5 mg for both alprazolam and triazolam) and to be rapidly absorbed after oral ingestion, although data from a self-experiment suggest that resorption can be delayed, for example because of food intake (Huppertz et al., 2018). Considering the pattern of halogen substitution, the elimination half-life of flubromazolam is likely to be in a range similar to that of the half-life of triazolam (approximately 2 hours) or the half-life of alprazolam (6–12 hours). Data from Huppertz et al. (2018) suggest that its half-life is approximately 10–20 hours. Although these data would be comparable to a short or medium duration of action, there were reports on long-lasting effects, in particular after ingestion of higher doses (Huppertz et al., 2018, Łukasik-Głębocka et al., 2016).

Manchester et al. (2018) experimentally determined the mean plasma protein binding (89.5 %) and pK_a (2.07) of flubromazolam. The in-silico predicted binding value (log 1/c) was 8.77 (Waters et al., 2018).

Noble et al. (2017) assessed plasma protein binding to be 89 %, by using an equilibrium dialysis assay, and found a hepatic clearance of 0.42 ml/minute/kg, which was between the values for diazepam (0.11 ml/minute/kg) and alprazolam (0.74 ml/minute/kg).

On TripSit (http://drugs.tripsit.me/flualprazolam, accessed October 2019) the following data are given, which probably originate from non-controlled self-experiments and have to be considered with caution: a common oral dose is given as 0.2–0.4 mg (threshold dose, 0.08 mg; light dose, 0.1–0.2 mg; strong dose, 0.4–0.6 mg). Onset of action is stated to occur 20–45 minutes after ingestion, duration of action is 6–12 hours and after-effects last 6–24 hours. A dose of about 0.25 mg of flubromazolam is stated to be equivalent to 10 mg of diazepam.

Toxicology

No data are available on the acute or chronic toxicity of flubromazolam, but, owing to the structural similarities to well-characterised triazolobenzodiazepines such as triazolam, toxicity can be anticipated to be similarly low.

Høiseth et al. (2016) detected flubromazolam in 25 out of 77 authentic forensic blood samples; the median concentration was 12 ng/ml (range 0.48–100 ng/ml). In one case with no other drugs detected, the male, a 19-year-old driver, was found to be considerably impaired at a blood concentration of 100 ng/ml.

Łukasik-Głębocka et al. (2016) reported on an intoxication associated with coma, respiratory depression and hypotension after the intake of a self-stated dose of 3 mg of flubromazolam. The patient improved after 4 days of treatment in hospital. Flumazenil (1.0 mg) temporarily improved the patient's vigilance for about 30 minutes. The reported flubromazolam serum concentration in a sample taken approximately 19 hours after drug intake was 43 ng/ml (Łukasik-Głębocka et al., 2016).

Huppertz et al. (2018) reported on a self-experiment comprising the ingestion of 0.5 mg of flubromazolam. The volunteer noticed muscle-relaxing effects and onset of a light tiredness approximately 90 minutes post ingestion. He seemed visibly impaired and experienced strong sedative effects and repeatedly fell asleep in the time interval from 3–10 hours after drug intake. In addition, partial amnesia extending beyond 24 hours was reported (Huppertz et al., 2018). Peak serum concentrations were about 8 ng/ml.

In 2019, Mei et al. published a validated LC-MS/MS method for the detection and quantification of 13 new benzodiazepines, including flubromazolam, in blood samples.

Several publications are dedicated to the metabolism of flubromazolam. Huppertz et al. (2015) described the formation of a monohydroxylated and a dihydroxylated metabolite after incubation with pooled human liver microsomes (pHLM). In addition, Pettersson Bergstrand et al. (2018) detected the glucuronides of flubromazolam and its monohydroxylated metabolites in the urine samples of patients. Wohlfarth et al. (2017) identified a total of seven metabolites by analysing authentic urine samples including two different glucuronides of flubromazolam, an *N*- and an *O*-glucuronide. Considering data from a self-administration study (oral, 0.5 mg of flubromazolam), Huppertz et al. (2018) suggested targeting the parent compound and the monohydroxylated metabolites in urine after enzymatic glucuronide hydrolysis, and showed that flubromazolam can be detected in hair after a low, single oral dose. Noble et al. (2017) showed that hydroxylation at the 4- and/or the α-position is catalysed mainly by CYP3A4 and (to a somewhat lower extent) CYP3A5, and confirmed the metabolite findings of the other groups.

Dependence and abuse potential

No data are available on the dependence and abuse potential of flubromazolam. However, it can be assumed that the abuse liability and the potential to produce dependence is similar to those of other chemically related triazolobenzodiazepines such as alprazolam or triazolam.

Epidemiology

Flubromazolam seems to be one of the most prevalent new benzodiazepines. It was frequently detected in a case series of patients with admitted or suspected intake of NPS presenting to hospitals in Sweden, covered as part of the STRIDA project (Bäckberg et al., 2019). More precisely, between 2012 and 2016, new benzodiazepine ingestion was analytically confirmed in 217 patients; in 42 % (n = 92) of these cases, flubromazolam was detected. Most of these cases occurred in 2015 (ranging from the end of 2014 to the beginning of 2016).

Carpenter et al. (2019) reported on 'designer benzodiazepine' exposures recorded in the United States between 2014 and 2017. Of 234 reported single-agent exposures to new benzodiazepines, 13 were flubromazolam cases (5.5 %). Two cases (3 %) were reported in 2016 and 11 cases (10 %) in 2017. In the majority of these cases (69 %), the effect duration was between 8 and 24 hours. The effects were minor or moderate in all cases.

In general, benzodiazepines play a major role in DUID offences, and flubromazolam has recently been reported to occur frequently in DUID cases involving new benzodiazepines (in 11 out of a total of 32 cases), which mainly occurred in the United States (UNODC, 2019).

Fluclotizolam

Background information

Fluclotizolam is a thienotriazolodiazepine that first appeared in 2017 as a new benzodiazepine and is structurally closely related to etizolam. It is thought to be about two to three times more potent than etizolam (a dose of 10 mg of diazepam is equivalent to about 1 mg of etizolam) and to have a somewhat shorter half-life.

Physical and chemical description

Fluclotizolam is a white powder in its pure form.

IUPAC name: 2-chloro-4-(2-fluorophenyl)-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]-diazepine.

CAS Number: 54123-15-8.

Sum formula: $C_{15}H_{10}ClFN_4S$.

Molecular mass: 332.7831 g/mol.

Monoisotopic mass: 332.0299 u; $[M+H]^+$: 333.0371 u.

Predicted log $D_{7.4}$ (using ACD/Labs Percepta Platform – PhysChem Module): 2.09 (http://www.chemspider.com/Chemical-Structure.15332007.html).

Pharmacology

To date, no data have been published in the scientific literature on the pharmacological properties of fluclotizolam. On TripSit (http://drugs.tripsit.me/fluclotizolam, accessed October 2019), the following data are given, which probably originate from non-controlled self-experiments and have to be considered with caution: a common oral dose is given as 0.25–0.5 mg (light dose, 0.25 mg; strong dose, 0.5–0.75 mg; heavy dose, ≥ 0.75 mg). Onset of action is stated to occur 10–30 minutes after ingestion, duration of action 3–6 hours and after-effects last for 1–14 hours.

Toxicology

No data are available on the acute or chronic toxicity of fluclotizolam, but owing to the structural similarities to the well-characterised thienotriazolodiazepine etizolam, toxicity can be anticipated to be similarly low.

Furthermore, no data have been published on the metabolism of fluclotizolam. However, it can be anticipated that hydroxylation catalysed by CYP enzymes occurs.

Dependence and abuse potential

No data are available on the dependence and abuse potential of fluclotizolam. However, it can be assumed that the abuse liability and the potential to produce dependence are similar to those of other chemically related thienotriazolodiazepines, such as etizolam.

Epidemiology

There are no available data on the epidemiology of fluclotizolam. Although included in the screening method since 2018 (about 500 samples analysed per year), at the time of writing it had not yet been detected in the laboratory of the Institute of Forensic Medicine in Freiburg (own unpublished data).

Diclazepam

Background information

Diclazepam, also known as Ro5-3448, is the 2-chloro derivative of diazepam and was first described by Sternbach et al. (1962). Its first appearance as a new benzodiazepine dates back to 2013. It seems to be several times more potent than diazepam.

Physical and chemical description

Diclazepam is a white powder in its pure form.

IUPAC name: 7-chloro-5-(2-chlorophenyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

CAS Number: 2894-68-0.

Sum formula: $C_{16}H_{12}Cl_2N_2O$.

Molecular mass: 319.1853 g/mol.

Monoisotopic mass: 318.0327 u; $[M+H]^+$: 319.0399 u.

Predicted log $D_{7.4}$ (using ACD/Labs Percepta Platform – PhysChem Module): 3.25 (http://www.chemspider.com/Chemical-Structure.68652.html).

Experimentally determined log $D_{7.4}$: 2.73 (Manchester et al., 2018).

Pharmacology

Babbini et al. (1979) showed that diclazepam is approximately four to eight times more potent than diazepam in terms of reducing motor activity and conflict behaviour in rats. Studies by Sternbach et al. (1968) found diclazepam to be equally as potent as diazepam with regard to muscle relaxant and sedative effects in mice and twice as potent than diazepam in cats. The K_i value at recombinant wild-type α1ß2γ2 $GABA_A$ receptors reflects a binding affinity that is 30 times higher than that of diazepam (Sigel et al., 1998). However, Bradley and Nicholson (1984) tested the behavioural activity of monkeys under diclazepam and diazepam (same dose) and did not see differences in the effects.

Manchester et al. (2018) experimentally determined the mean plasma protein binding (93.8 %) and the pK_a value (2.31) of diclazepam. The in-silico predicted binding value (log 1/c) was 8.39 (Waters et al., 2018).

On TripSit (http://drugs.tripsit.me/diclazepam, accessed October 2019), the following data are given, which probably originate from non-controlled self-experiments and have to be considered with caution: a common oral dose is given as 1–2 mg (light dose, 0.25–1 mg; strong dose, ≥ 2 mg). Onset of action is stated to occur 15–90 minutes after ingestion, duration of action lasts 8–12 hours and after-effects last 1–24 hours. A dose of about 1 mg of diclazepam is stated to be equivalent to 10 mg of diazepam (considering the available data, potency tends to be somewhat overestimated here).

Toxicology

No data are available on the acute or chronic toxicity of diclazepam, but owing to the structural similarities to the well-characterised 1,4-benzodiazepines delorazepam, lorazepam and lormetazepam, which are metabolites of diclazepam, toxicity can be anticipated to be similarly low.

Moosmann et al. (2014) investigated the metabolism of diclazepam and performed a self-experiment of oral ingestion of 1 mg of the compound. The maximum serum concentration was 3.4 ng/ml and the subject did not notice any pharmacological effect. They reported an elimination half-life of approximately 42 hours. The pharmacologically active metabolites lorazepam and lormetazepam have shorter elimination half-lives (12 and 13 hours respectively) and delorazepam has a half-life of about 78 hours.

In 2019, Mei et al. published a validated LC-MS/MS method for the detection and quantification of 13 new benzodiazepines, including diclazepam, in blood samples.

Høiseth et al. (2016) detected diclazepam in 15 out of 77 authentic forensic blood samples, and the median concentration was 13 ng/ml (range 2.1–57 ng/ml). In one case with no other drugs detected, the male, an 18-year-old driver, was found to be considerably impaired at a blood concentration of 57 ng/ml.

Dependence and abuse potential

No data are available on the dependence and abuse potential of diclazepam. However, it can be assumed that the abuse liability and the potential to produce dependence are similar to those of other chemically related 1,4-benzodiazepines, such as diazepam.

Epidemiology

Diclazepam was analytically confirmed in four cases of a case series of consecutive patients with admitted or suspected intake of NPS presenting to hospitals in Sweden between 2012 and 2016, as part of the STRIDA project (Bäckberg et al., 2019) (1.8 % of all cases with the detection of at least one new benzodiazepine, of which most occurred between the end of 2014 and the beginning of 2015).

Carpenter et al. (2019) reported on 'designer benzodiazepine' exposures recorded in the United States between 2014 and 2017. Of 234 reported single-agent exposures to new benzodiazepines, four were diclazepam cases (1.7 %). One case was reported in 2016 and three cases (2.7 %) in 2017.

In general, benzodiazepines play a major role in DUID offences, and diclazepam has recently been reported in one case of a DUID case series involving new benzodiazepines (total number: 32 cases), which mainly occurred in the United States (UNODC, 2019).

Clonazolam/clonitrazolam

Background information

Clonazolam was first described in an article written by Hester et al. in 1971; it was described as the most active compound in the investigated series of 16 6-phenyl-4*H*-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine derivatives, being effective in many tests at doses of less than 10 µg/kg (Hester et al., 1971). The first appearance of clonazolam as a new benzodiazepine available via internet shops was in 2014.

Physical and chemical description

Clonazolam is a white powder in its pure form.

IUPAC name: 6-(2-chlorophenyl)-1-methyl-8-nitro-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine.

CAS Number: 33887-02-4.

Sum formula: $C_{17}H_{12}ClN_5O_2$.

Molecular mass: 353.7625 g/mol.

Monoisotopic mass: 353.0680 u; $[M+H]^+$: 354.0752 u.

Melting point: 229–231 °C (Hester et al., 1971).

Predicted log $D_{7.4}$ (using ACD/Labs Percepta Platform – PhysChem Module): 2.24 (http://www.chemspider.com/Chemical-Structure.15468596.html).

Pharmacology

Hester et al. (1971) described clonazolam as the most potent compound of a series of chemically similar benzodiazepines.

The in-silico predicted binding value (log 1/c) was given as 8.86, which was the second highest value after flunitrazolam (8.86), confirming the extremely high potency of this compound (Waters et al., 2018).

On TripSit (http://drugs.tripsit.me/clonazolam, accessed October 2019), the following data are given, which probably originate from non-controlled self-experiments and have to be considered with caution: a common oral dose is given as 0.2–0.4 mg (threshold dose, 0.05–0.075 mg; light dose, 0.075–0.2 mg; heavy dose, 0.5–1 mg). Onset of action is stated to occur 10–30 minutes after ingestion, duration of action is 6–10 hours and after-effects last 1–12 hours.

Toxicology

No data are available on the acute or chronic toxicity of clonazolam, but, owing to the structural similarities to

the well-characterised triazolobenzodiazepine triazolam and the nitrobenzodiazepine clonazepam, toxicity can be anticipated to be similar to these established benzodiazepines.

In 2019, Mei et al. published a validated LC-MS/MS method for the detection and quantification of 13 new benzodiazepines, including clonazolam, in blood samples.

Høiseth et al. (2016) detected clonazolam in 7 out of 77 authentic forensic blood samples; the median concentration was 5.3 ng/ml (range 1.9–11 ng/ml).

Data on the *in vitro* metabolism of clonazolam after incubation with pHLM were published by Huppertz et al. (2015); they reported a monohydroxylated metabolite and a reduction of the amino function leading to 8-aminoclonazolam. Meyer et al. (2016) reported a total of seven metabolites in human urine samples with 8-aminoclonazolam, 8-acetamidoclonazolam and one monohydroxylated metabolite (probably α-position) being the main phase I metabolites, followed by another monohydroxylated metabolite (4-position) and three glucuronides. The parent compound was detected in all authentic urine samples (n = 4), although in relatively low concentrations. El Balkhi et al. (2017) confirmed the results of Huppertz et al. (2015) after pHLM incubation and tentatively identified a further metabolite corresponding to a demethylation.

Dependence and abuse potential

No data are available on the dependence and abuse potential of clonazolam. However, it can be assumed that the abuse liability and the potential to produce dependence are similar to those of other chemically related and highly potent triazolobenzodiazepines, such as triazolam.

Epidemiology

Clonazolam was analytically confirmed in 16 cases of a case series of consecutive patients with admitted or suspected intake of NPS presenting to hospitals in Sweden between 2012 and 2016, as part of the STRIDA project (Bäckberg et al., 2019) (7.4 % of all cases with the detection of at least one new benzodiazepine). These cases mainly occurred in 2015.

Carpenter et al. (2019) reported on 'designer benzodiazepine' exposures recorded in the United States between 2014 and 2017. Of 234 reported single-agent exposures to new benzodiazepines, 50 were clonazolam cases (21 %). Fourteen cases (21 %) were reported in 2016 and 36 cases (32 %) in 2017. In the majority of these cases (46 %), the effect duration was between 8 and 24 hours. The effects were minor or moderate in all cases but two, which showed 'major effects'.

In general, benzodiazepines play a major role in DUID offences, and clonazolam has recently been reported to have occurred in DUID cases (5 out of 32 cases testing positive for at least one new benzodiazepine), which mainly occurred in the United States (UNODC, 2019).

N-Desalkylflurazepam (norflurazepam)

Background information

N-Desalkylflurazepam was first mentioned in a patent granted to Hoffmann-La Roche Inc. in 1976. It is a long-acting 1,4-benzodiazepine and is known as the main metabolite of flurazepam. It also occurs as a metabolite of some other benzodiazepines (e.g. fludiazepem, flutoprazepam and quazepam) and was shown to be an impurity present in midazolam medicines (Vogt et al., 2013). It first appeared as a new benzodiazepine available online in 2016.

Physical and chemical description

N-Desalkylflurazepam is a white powder in its pure form.

IUPAC name: 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-1,4-benzodiazepin-2-one.

CAS Number: 2886-65-9.

Sum formula: $C_{15}H_{10}ClFN_2O$.

Molecular mass: 288.7041 g/mol.

Monoisotopic mass: 288.0466 u; $[M+H]^+$: 289.0538 u.

Melting point: 205–207 °C (Chase, 1976)

Predicted log $D_{7.4}$ (using ACD/Labs Percepta Platform – PhysChem Module): 2.80 (http://www.chemspider.com/Chemical-Structure.4381.html).

Experimentally determined log $D_{7.4}$: 2.82 (Manchester et al., 2018).

Pharmacology

Manchester et al. (2018) experimentally determined the mean plasma protein binding (95.5 %), pK_a (2.51) and log $D_{7.4}$ (2.82) of *N*-desalkylflurazepam. The in-silico predicted binding value (log 1/c) was 8.44 (Waters et al., 2018).

On TripSit (http://drugs.tripsit.me/norflurazepam, accessed October 2019) the following data are given, which probably originate from non-controlled self-experiments and have to be considered with caution: a common oral dose is given as 5–10 mg (light dose, 2–5 mg; strong dose, 10–20 mg or more). Onset of action is stated to occur 45–120 minutes after ingestion, duration of action is 10–16 hours and after-effects last 1–12 hours.

Toxicology

No specific data are available on the acute or chronic toxicity of *N*-desalkylflurazepam. However, *N*-desalkylflurazepam is the main metabolite of flurazepam and accumulates after continued flurazepam medication. Therefore, toxicity can be anticipated to be similarly as low as for flurazepam.

The LD_{50} in mice after oral administration was determined to be 2 250 mg/kg (Garattini et al., 1973). The elimination half-life of *N*-desalkylflurazepam was given as 74 hours ± 24 hours by Schulz et al. (2012), but the original literature was not cited. Greenblatt et al. (1983) reported an elimination half-life of 40–200 hours, which could potentially lead to extensive accumulation after repeated intake.

Moosmann et al. (2019) detected two hydroxylated (hydroxylation was hypothesised to occur at carbon atoms 3 and 4') and one dihydroxylated metabolites of norflurazepam after incubation with pHLM.

Dependence and abuse potential

No data are available on the dependence and abuse potential of *N*-desalkylflurazepam. However, it can be assumed that the abuse liability and the potential to produce dependence are similar to those of other chemically related benzodiazepines, such as flurazepam.

Epidemiology

No data are available on the epidemiology of *N*-desalkylflurazepam. Assessing the prevalence of *N*-desalkylflurazepam is complicated by the fact that it occurs as an accumulating metabolite of several other benzodiazepines.

Carpenter et al. (2019) reported on 'designer benzodiazepine' exposures recorded in the United States between 2014 and 2017. Of a total of 234 reported single-agent exposures to new benzodiazepines, only one case was reported as a norflurazepam intoxication; that case occurred in 2017.

Bromazolam

Background information

Bromazolam is the bromo analogue of alprazolam and was first described by Hester and Von Voigtlander in 1979 (Hester and Von Voigtlander, 1979). Its first appearance as a new benzodiazepine dates back to 2016.

Physical and chemical description

Bromazolam is a white powder in its pure form.

IUPAC name: 8-bromo-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine.

CAS Number: 71368-80-4.

Sum formula: $C_{17}H_{13}BrN_4$.

Molecular mass: 353.2159 g/mol.

Monoisotopic mass: 352.0324 u; $[M+H]^+$: 353.0396 u.

Melting point: 272–272.5 °C (Hester and Von Voigtlander, 1979).

Predicted log $D_{7.4}$ (using ACD/Labs Percepta Platform – PhysChem Module): 1.86 (http://www.chemspider.com/Chemical-Structure.19871738.html).

Pharmacology

Bromazolam was investigated pharmacologically by Clayton et al. (2015) under the name XLi268 and showed K_i values in the low to sub-nanomolar range at different $GABA_A$ receptors.

On TripSit (http://drugs.tripsit.me/bromazolam, accessed October 2019), the following data are given, which

probably originate from non-controlled self-experiments and have to be considered with caution: a common oral dose is given as 1–3 mg (light dose, 0.5–1 mg; strong dose, 3–5 mg or more). Onset of action is stated to occur 15–45 minutes after ingestion, duration of action is 5–8 hours and after-effects last 1–12 hours.

Toxicology

No specific data are available on the acute or chronic toxicity of bromazolam, but, owing to the structural similarity to the well-characterised triazolobenzodiazepine alprazolam, toxicity can be anticipated to be similarly low.

Dependence and abuse potential

No data are available on the dependence and abuse potential of bromazolam. However, it can be assumed that the abuse liability and the potential to produce dependence are similar those of to other chemically related benzodiazepines, such as alprazolam.

Epidemiology

No data are available on the epidemiology of bromazolam.

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emcdda

About this publication

This report provides a technical review of the current body of knowledge regarding new benzodiazepines that are monitored by the EU Early Warning System. The aims of this report are to strengthen situational awareness of new benzodiazepines in Europe, and to help stakeholders prepare for and respond to public health and social threats caused by such substances.

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