Benzodiazepines

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Abstract

Benzodiazepines are the most widely prescribed central nervous system (CNS) depressants in the United States. They have been in use since the 1960s as anxiolytics, anticonvulsants, and muscle relaxants. The name of the class is derived from the combination of a benzene ring with a seven-membered diazepine ring. Included in the structure is a phenyl ring attached to the 5-position of the diazepine, a requirement for benzodiazepine activity. Benzodiazepines mediate their CNS-depressant activity through the neurotransmitter gamma-amino butyric acid (GABA), the major inhibitory neurotransmitter in the brain. Benzodiazepines bind to $GABA_A$ receptors and potentiate the inhibitory action of GABA. Benzodiazepines have a high therapeutic index and, as a result, rarely cause death by themselves. In addition to the widely prescribed benzodiazepines such as alprazolam, clonazepam, diazepam, and lorazepam, a number of analogs have been developed illicitly, including pyrazolam, flubromazolam, and others. Analysis of benzodiazepines often requires the inclusion of metabolites, depending on the specimen being tested. The chemistry, pharmacology, effects, and analysis of therapeutic and designer benzodiazepines are reviewed.

Keywords

Benzodiazepines · Pharmacology · Effects · Toxicology · Interpretation · Analysis

As a class, benzodiazepines are one of the most widely prescribed drugs in the world and have largely replaced barbiturates as the major class of CNS-depressant drugs. Data from 2018 indicated that five benzodiazepines rank among the top 200 drugs in the United States, as determined by the number of prescriptions dispensed. These top-ranking benzodiazepines were alprazolam (#23), clonazepam (#38), lorazepam (#55), diazepam (#91), and temazepam (#174). Currently, approximately 24 benzodiazepines, including prodrugs, are approved for use in the United States and are prescribed as anxiolytics, muscle relaxants, anesthetic adjuncts, anticonvulsants, and treatment for obsessive-compulsive disorders. The properties of selected benzodiazepines are summarized in Table 20.1.

Dr. Leo Sternbach of Hoffmann-La Roche is credited with the discovery of the benzodiazepines. During an assistantship in Poland in the 1930s, Dr. Sternbach studied a class of

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Table 20.1 Selected properties of common benzodiazepines

Benzodiazepine	Trade name(s)	Uses	Primary metabolite(s)	Half-life	V_d	Dose	Therapeutic concentrations
Alprazolam	Xanax	Antidepressant Anxiolytic	α-Hydroxyalprazolam	11–15 h	0.7–1.3 L/kg	General anxiety: 0.75–4 mg/day Panic disorder: Up to 10 mg/day	5–50 ng/mL
Bromazepam	Lectopam Lexotan	Anxiolytic Muscle relaxant	3-Hydroxybromazepam glucuronide 2-Amino-5-bromo-3-hydroxy-benzoylpyridine glucuronide	8–19 h	0.9 L/kg	3–18 mg daily (max 60 mg daily)	0.08–0.15 mg/L
Chlordiazepoxide	Librium	Anxiolytic Alcohol withdrawal	Norchlordiazepoxide	5–30 h (CDP)	0.3–0.6 L/kg	Anxiety: 30–100 mg daily	0.4–4 mg/L
			Demoxepam Nordiazepam Oxazepam	30–100 h (nordiazepam)		Alcohol withdrawal: Up to 300 mg daily	
Clobazam	Frisium Urbanyl	Anticonvulsant Anxiolytic Panic disorder Sedative	Desmethylclobazam 2–3 d (desmethylclobazam)	10–30 h (clobazam)	0.9–1.8 L/kg	20–60 mg daily	0.1–0.4 mg/L
Clonazepam	Klonopin	Anticonvulsant Panic disorder	7-Aminoclonazepam	19–60 h	2–4 L/kg	1.5–20 mg daily	0.005–0.07 mg/L
Clorazepate	Tranxene	Anticonvulsant Anxiolytic Alcohol withdrawal	Nordiazepam Oxazepam	2 h (clorazepate) 30–100 h (nordiazepam)	0.5–2.5 L/kg	Up to 60 mg daily	0.02–0.8 mg/L nordiazepam
Diazepam	Valium	Anticonvulsant Anxiolytic Muscle relaxant	Nordiazepam	20–50 h (diazepam) 30–100 h (nordiazepam)	0.5–2.5 L/kg	2–40 mg daily	0.1–1.5 mg/L
Estazolam	ProSom	Hypnotic	1-Oxoestazolam	10–30 h	3 L/kg	1–2 mg	0.05–0.1 mg/L
	Eurodin		4-Hydroxyestazolam				
Flunitrazepam	Rohypnol	Anesthetic induction agent	7-Aminoflunitrazepam	9–25 h	3.5–5.5 L/kg	0.5–2 mg	0.005–0.015 mg/L
		Hypnotic					

Flurazepam	Dalmane	Hypnotic	N-1-Desalkylflurazepam N-1-Hydroxyethyl-flurazepam	1–3 h (flurazepam)	3.4–5.5 L/kg 80 h (N-1-desalkylflurazepam)	15–30 mg daily	0.0005– 0.03 mg/L
Halazepam	Paxipam	Anxiolytic	Nordiazepam	14–16 h	1.0 L/kg	20–40 mg 3–4× daily	0.037– 0.125 mg/L
Lorazepam	Ativan	Anxiolytic Pre-operative	Lorazepam glucuronide	9–24 h	1–2 L/kg	1–10 mg daily	0.05–0.24 mg/L
Midazolam	Versed	Anesthetic induction agent Pre-operative Sedative	α-Hydroxymidazolam	1.5–2.5 h	1.0–2.5 L/kg	0.05–0.5 mg/kg	0.08–0.25 mg/L
Nitrazepam	Mogadon	Hypnotic	7-Aminonitrazepam	16–48 h	2–5 L/kg	5–10 mg daily	0.03–0.12 mg/L
Oxazepam	Serax	Anxiolytic	Oxazepam glucuronide	4–15 h	0.5–2.0 L/kg	15–60 mg daily	0.5–2.0 mg/L
Prazepam	Centrax	Anxiolytic	Nordiazepam	1.3 h (Prazepam)	12–14 L/kg	20–60 mg daily	0.02–0.8 mg/L
				30–100 h (Nordiazepam)			Nordiazepam
Temazepam	Normison	Hypnotic	Oxazepam	5–15 h	0.8–1.4 L/kg	15–60 mg daily	0.3–0.9 mg/L
	Restoril	Pre-operative					
Triazolam	Halcion	Hypnotic	α-Hydroxytriazolam	1.5–5.5 h	1.1–2.7 L/kg	0.125–0.25 mg daily	0.002–0.02 mg/L

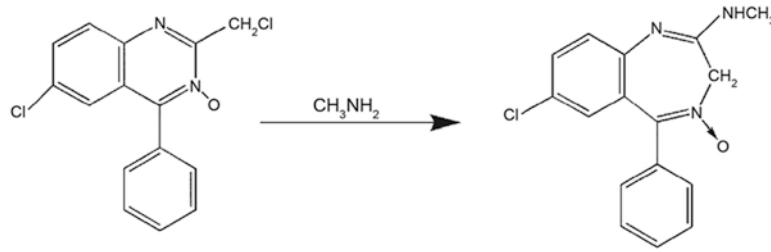


Fig. 20.1 The first benzodiazepine was synthesized by the reaction of a quinazoline N-oxide with methylamine

compounds called heptoxdiazines. His interest in these compounds resurfaced in the mid-1950s when he began to evaluate "heptoxdiazines" as potential tranquilizers. During his investigation, he discovered that these compounds were not heptoxdiazines as previously thought, but they were quinazoline 3-oxides. After several years of synthesizing numerous quinazoline 3-oxide compounds with disappointing pharmacological test results, Dr. Sternbach initiated a "cleanup" of the laboratory and expected to complete this work with at least some publishable material. During the cleanup, a co-worker drew his attention to a compound that formed when quinazoline N-oxide was treated with methylamine. This compound was subsequently submitted for animal pharmacological testing, which yielded promising results. Further testing indicated that this compound was the product of an unusual ring enlargement, which had created a benzodiazepine derivative (Fig. 20.1). Clinical trials with this benzodiazepine compound were initiated in 1958, leading to its approval by the FDA in February 1960. One month later it was marketed as Librium (chlordiazepoxide), the first benzodiazepine approved for therapeutic use.

Chemistry and Use

The general benzodiazepine structure is shown in Fig. 20.2. The name of the class is derived from the combination of a benzene ring (A) with a seven-member diazepine ring (B). Included in the structure is a phenyl ring (C) attached to the 5-position of the diazepine ring. This phenyl group (C) appears to be a requirement for benzodiazepine activity. Moreover, the benzene component of the benzodiazepine structure needs an electron-withdrawing group present at R7 to have enhanced activity; a chlorine atom and a nitro group are the most common entities attached. Potency can be improved by adding an electron-withdrawing group in the ortho position (R2') on the phenyl ring attached to the benzodiazepine nucleus (i.e., lorazepam, clonazepam, flunitrazepam). An electron-withdrawing group at this position also produces a greater amnesic effect.

Other structural modifications are possible and can affect both potency and duration of action. Groups commonly bonded to the N1 position include hydrogen, a substituted alkyl group, or a fused triazole (triazolobenzodiazepines) or imidazole ring (imidazobenzodiazepines). Benzodiazepines with smaller substituents on position N1 tend to have higher intrinsic activity; however, some drugs with larger N1 substituents are effective (e.g., flurazepam), largely due to metabolic dealkylation to an active metabolite. Most benzodiazepines have a double-bonded oxygen attached to C2 (the exceptions are chlordiazepoxide, which has a methylamino group, and quazepam, which has a double-bonded sulfur). Replacing a hydrogen with a hydroxyl group on C3 reduces the drug's duration of action. The influence of these structural changes on the pharmacological effects can be exploited for a wide variety of clinical purposes. The addition of the hydroxyl group allows direct conjugation of the parent drug to an inactive glucuronide conjugate. An additional ring can be added to positions 1 and 2 of the diazepine ring, resulting in the highly potent imidazo- (e.g., midazolam) and triazolobenzodiazepines (e.g., triazolam). A host of analogs are possible, and this has been exploited for illicit purposes. Some of the newer ("designer") benzodiazepines involve the replacement of the phenyl ring (C) with a pyridine (e.g., pyrazolam)

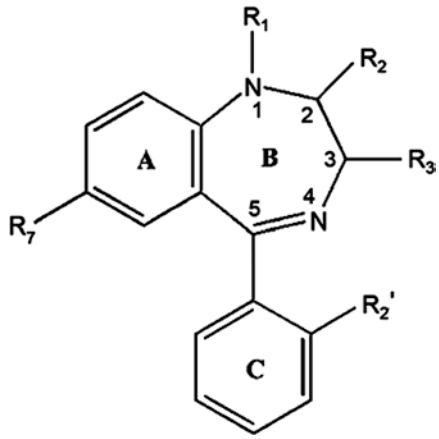


Fig. 20.2 The benzodiazepine structure

or replacement of the benzene ring (A) with thiophene ring (e.g., etizolam). Selected benzodiazepines are illustrated in Figs. 20.3 and 20.4.

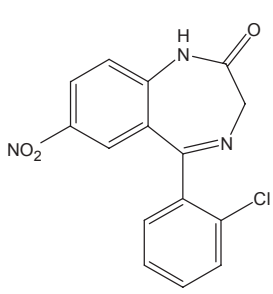
As therapeutic agents, benzodiazepines have many advantages over the drugs they largely replaced, barbiturates; namely, they have fewer side effects and are much safer from the standpoint of potential overdose. Benzodiazepines display selective rather than generalized central nervous system depressant actions. This gives them a uniquely wide margin of safety. There is less liver enzyme induction with benzodiazepines, which presents fewer complications when multiple drugs are co-administered. While withdrawal effects after discontinuing benzodiazepine use do occur, the symptoms are milder than those observed with barbiturates.

Benzodiazepines have wide-ranging clinical utility. One of the most common therapeutic uses of benzodiazepines is to treat anxiety. Diazepam gained widespread use in the 1970s as an anxiolytic drug. Alprazolam was introduced in the US pharmaceutical market in the 1980s and has largely replaced diazepam for this purpose. The anxiolytic effects of benzodiazepines are likely related to their ability to produce inhibitory effects in areas of the brain that are associated with anxiogenesis. Additionally, it has been reported that benzodiazepines act to suppress noradrenergic and/or serotonergic pathways in some areas of the brain, which appears to play a role in their anxiolytic effects. The major advantages of benzodiazepines as anxiolytics include their rapid onset of action and their safety. The major disadvantages of benzodiazepines as anxiolytics include the development of tolerance and/or dependence with long-term use and their potential negative effects on psychomotor performance.

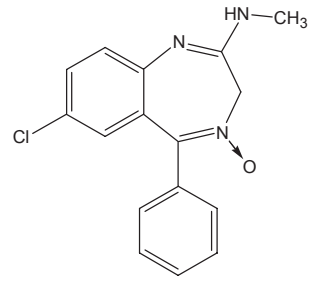
Benzodiazepines are also prescribed as hypnotic agents for the treatment of insomnia. Insomnia is a fairly common condition, affecting 30–40% of the US adult population within a given year. It is more common in women and its prevalence increases with age. To be an effective hypnotic, a drug should have a rapid onset of action, assuming that it is taken at bedtime. Ideally, the duration of action should be long enough to allow a complete night's sleep but not so long that the drowsiness persists into the following day (the hangover effect). Benzodiazepines effectively treat insomnia; their use often results in a more rapid sleep onset, decreased nighttime awakenings, and an increased total sleeping time. However, benzodiazepine-induced sleep differs from natural sleep, resulting in prolonged periods of light sleep and decreased duration of (rapid eye movement) REM and slow wave sleep. The major disadvantages associated with benzodiazepine hypnotics include the development of tolerance and dependence, rebound insomnia with discontinuation of use, hangover effects, and respiratory depression that can aggravate some respiratory conditions.

The benzodiazepines are also used clinically for the management of seizure disorders. For example, diazepam has long been the drug of choice for the treatment of status epilepticus. Clonazepam can be used to treat a variety of seizures (with the exception of generalized tonic-clonic seizures). The major disadvantages of benzodiazepines as anti-epileptics are the development of tolerance in many patients and potential sedation and psychomotor impairment.

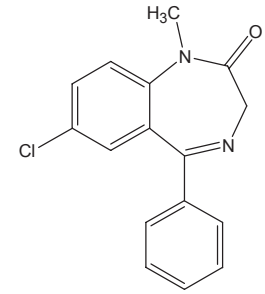
In addition to their use as therapeutic agents, benzodiazepines produce sedative and amnesic effects for brief medical procedures and are used to premedicate patients. An intravenous dose of a short-acting benzodiazepine, midazolam, assists in the induction of surgical anesthesia. Diazepam



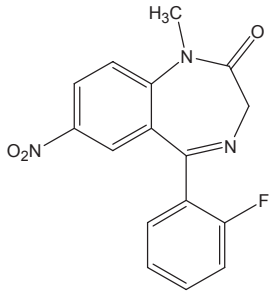
Clonazepam



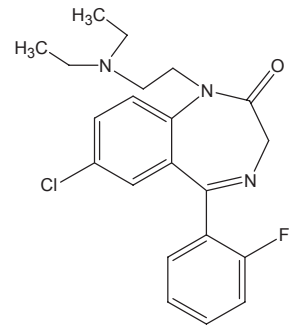
Chlordiazepoxide



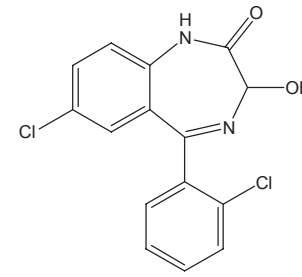
Diazepam



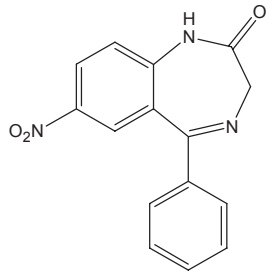
Flunitrazepam



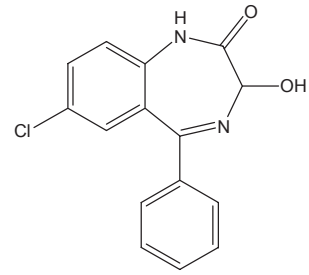
Flurazepam



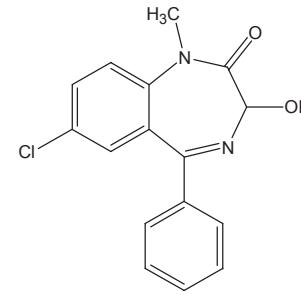
Lorazepam



Nitrazepam

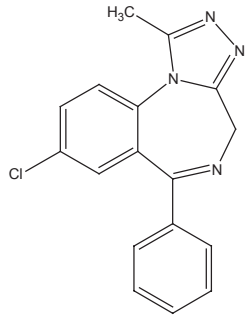


Oxazepam

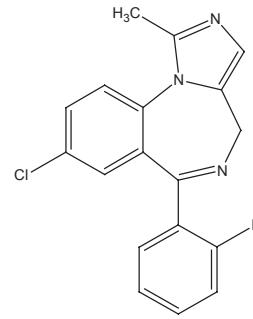


Temazepam

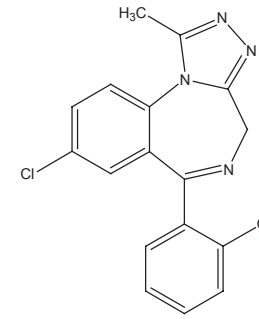
Fig. 20.3 Structures of selected 1,4-benzodiazepines



Alprazolam



Midazolam



Triazolam

Fig. 20.4 Structures of selected imidazo- and triazolobenzodiazepines

and lorazepam are also administered as preanesthetic medications. Diazepam is an effective muscle relaxant. It has been used in this capacity to treat various motion disorders. Chlordiazepoxide and diazepam have been used to treat alcohol dependence. They are administered during alcohol detoxification to prevent seizures associated with withdrawal.

Benzodiazepines are often used in combination with illicit drugs. The combination of benzodiazepines with opiates has been reported to produce an enhanced high. Benzodiazepines in combination with cocaine decrease the seizure threshold and are reported to offset the negative effects of the drug following binge use. Benzodiazepines may also reduce withdrawal symptoms associated with drugs of abuse. As a consequence, they are frequently detected in combination with illicit drugs. The most commonly encountered benzodiazepines among illicit drug users are diazepam and alprazolam.

When benzodiazepines are prescribed as therapeutic agents, they are recommended for short-term (4 weeks or less) or intermittent use in most cases, since tolerance and dependence can occur with extended use. Tolerance to the sedative effects of benzodiazepines develops rapidly, usually within a week of the initiation of benzodiazepine therapy. However, tolerance to the anxiolytic effects appears to develop more slowly and to a lesser degree. Long-term benzodiazepine use has been associated with dependence. It has been reported that approximately 35% of patients taking benzodiazepines for more than 4 weeks—regardless of whether the dosage is therapeutic or excessive—develop dependence, as evidenced by the appearance of withdrawal symptoms following dosage decrease or termination. In addition, patients who are on benzodiazepines for extended periods (without dose escalation) may exhibit withdrawal symptoms such as anxiety, agitation, irritability, increased sensitivity to light and sound, muscle cramps, myoclonic jerks, insomnia, fatigue, headache, dizziness, concentration difficulties, paresthesias, nausea, seizures, loss of appetite, weight loss, and depression. Benzodiazepines with higher potency and shorter elimination half-lives appear to be associated with an increased risk of dependence.

Although benzodiazepines are considered relatively safe drugs, overdose can produce life-threatening effects. When benzodiazepine intoxication occurs, it can be treated with a variety of measures, including generalized supportive care, monitoring of vital signs, maintenance of adequate airway, and administration of vasopressors to treat hypotension. Activated charcoal administration is most beneficial within two to four hours of ingestion and when risk of aspiration is minimal. Flumazenil, a GABA antagonist, may be administered if appropriate. However, flumazenil must be used with caution because it reduces seizure threshold and may actually precipitate seizure activity in a patient who has co-ingested a substance that induces seizures (e.g., tricyclic antidepressants) or in a patient with an underlying seizure disorder. When a patient presents with suspected benzodiazepine intoxication, it is important to identify any co-intoxicants, as they may alter the recommended course of treatment.

Although benzodiazepines can be classified according to their chemical structure (described above), they are also classified according to their pharmacological properties, namely, their duration of action and elimination half-lives.

Pharmacology

Mechanism of Action

Benzodiazepines mediate their CNS-depressant activity through the neurotransmitter gamma-amino butyric acid (GABA). GABA is the major inhibitory neurotransmitter in the brain and consists of two subtypes: (1) $GABA_A$ and (2) $GABA_B$.

$GABA_A$ receptors are a set of ligand-gated ion channels that convey GABA's effect on fast synaptic transmission. Benzodiazepines bind to $GABA_A$ receptors and potentiate the inhibitory action of GABA. Activating the $GABA_A$ receptor opens an ion channel and allows chloride ions to enter the cell. As a result, neuronal activity slows down because of hyperpolarization of the cell

membrane potential. Specifically, the binding of the drug increases the amount of chloride current generated by the $GABA_A$ receptor complex, increasing inhibitory effect. Benzodiazepine binding to the $GABA_A$ receptor does not open the chloride ion channel directly, but increases the effectiveness of GABA by decreasing the concentration of GABA required to open the channel. There are also multiple subtypes of $GABA_A$ receptors, and benzodiazepines appear to interact with many of these subtypes. This accounts for the varied pharmacologic uses of the drugs.

Pharmacokinetics

Benzodiazepines may be administered orally, intravenously, or intramuscularly. When taken orally, they are completely absorbed—their high lipid solubility aids absorption. However, the rate of absorption depends on the benzodiazepine. For example, diazepam reaches peak blood concentrations within an hour after ingestion. Other benzodiazepines require several hours to reach their peak. Several benzodiazepines, such as clorazepate and prazepam, serve as prodrugs, being rapidly broken down to nordiazepam, which is the active drug.

Generally, benzodiazepines display a significant first-pass effect prior to general distribution. Their volume of distribution is around 2 L/kg and they are highly protein-bound. Typically, the fraction-bound portion is >80%. The major binding protein for benzodiazepines is albumin; however, it appears that triazolobenzodiazepines bind to all acid glycoprotein. Like barbiturates, benzodiazepines are classified according to their elimination half-lives. Midazolam and triazolam are considered short-acting because their elimination half-lives are only several hours. Many (such as alprazolam, lorazepam, oxazepam, and temazepam) have elimination half-lives of 6–24 h and are classified as intermediate-acting. Long-acting benzodiazepines, such as diazepam and quazepam, have elimination half-lives >24 h. One difficulty in the establishment of this classification is the presence of active metabolites that may have substantially different half-lives than the parent drug. For example, flurazepam has an elimination half-life of several hours, but an active metabolite has an elimination half-life of several days. Selected properties of the benzodiazepines are summarized in Table 20.1.

Benzodiazepines are extensively metabolized in the liver. Microsomal enzymes play a prominent role in this metabolism. Phase I metabolic routes include hydroxylation, dealkylation, deamination, and reduction. Substituents are usually removed from the B-ring, with larger or further removed alkyl substituents often removed more rapidly than smaller ones. Hydroxylation, a slow process that can take about 100 hours, usually occurs at position R3 of the B-ring. Structural modifications, such as the presence of a pyridine ring (e.g., bromazepam) can greatly enhance the rate of hydroxylation. The cytochrome P450 3A3 and 3A4 enzyme subtypes mediate many hydroxylation and dealkylation reactions.

Table 20.2 gives specific examples of each type of metabolic pathway. As previously stated, many of these metabolites have CNS-depressant activity that affects potency and duration of action. Once hydroxyl products are formed, phase II metabolism or conjugation with glucuronic acid then occurs. These conjugated metabolites are the major urinary products of benzodiazepines.

Performance Effects of Benzodiazepines

As central nervous system depressants, benzodiazepines can have significant impairing effects, even at prescribed doses. Such effects include prolonged reaction times, impaired judgment, impaired coordination, decreased alertness and concentration, and impaired short-term memory. Clinical studies have demonstrated that typical doses of diazepam, nitrazepam, flunitrazepam, flurazepam, lorazepam, and triazolam can impair some skills necessary for driving. Concomitant use of ethanol and benzodiazepines will increase impairment. Tolerance to some of these effects can develop with prolonged use. However, considering the scale on which benzodiazepines

Table 20.2 Examples of benzodiazepine phase I metabolism

Reaction	Precursor	Product
Dealkylation		
	Diazepam	Nordiazepam
	Temazepam	Oxazepam
	Flurazepam	N-1-desalkylflurazepam
Deamination		
	Chlordiazepoxide	Demoxepam
Hydroxylation		
	Alprazolam	α-Hydroxyalprazolam
	Diazepam	Temazepam
	Nordiazepam	Oxazepam
Reduction		
	Clonazepam	7-Aminoclonazepam
	Demoxepam	Nordiazepam

are prescribed, their effects on driving skills and potential contribution to traffic and other accidents are a major concern.

Interactions

When administered alone, benzodiazepines are relatively safe drugs. There are few reports of fatal overdoses due solely to benzodiazepine toxicity. However, benzodiazepine use in combination with other CNS depressants can increase toxicity: recent Drug Abuse Warning Network data indicated that 78% of benzodiazepine-related emergency department visits involved two or more drugs. The drugs most often combined with benzodiazepines were alcohol, illicit drugs, and opiates.

Since the cytochrome P450 3A enzyme family is involved in the metabolism of many benzodiazepines, it is important to consider the potential effects of drugs that induce or inhibit this enzyme system. Some of the more commonly encountered drugs that inhibit the CYP3A enzymes include cimetidine, diltiazem, fluoxetine, fluvoxamine, paroxetine, verapamil, antifungals, and protease inhibitors. Co-administration of some of these inhibitors and benzodiazepines has been reported to produce clinically significant effects, including increased blood benzodiazepine concentrations and increased benzodiazepine elimination half-life. Drugs that induce the CYP3A enzyme family include barbiturates, phenytoin, carbamazepine, and rifampicin. Drugs that alter glucuronyl transferase activity may also affect the metabolism of 3-hydroxy benzodiazepines.

Special Considerations

There is limited data on the effects of benzodiazepines on the fetus and nursing infants. The available data suggest that benzodiazepine administration during pregnancy does not increase the risk of congenital malformations. However, the data are insufficient to definitively state that there is no risk of injury to the fetus with benzodiazepine exposure. Withdrawal can occur in infants of mothers receiving chronic benzodiazepine therapy, especially if benzodiazepines are administered near term or during delivery. Although benzodiazepines are not contraindicated in lactating mothers, there is evidence that some benzodiazepines are excreted into breast milk, at concentrations about 10–20% of plasma concentrations. If benzodiazepines are administered to mothers who breastfeed, their infants should be closely watched for lethargy, sedation, and weight loss.

Benzodiazepines are widely administered to the elderly. It has been estimated that elderly patients receive 50% of all benzodiazepine prescriptions, although they account for less than 13% of the population. Studies have indicated that the elderly show increased sensitivity to the effects of some benzodiazepines. This is partially due to the decrease in the rate at which the elderly

oxidize some benzodiazepines. The decreased rate of metabolism is a result of the decreased CYP3A4 activity that occurs with age. The pharmacokinetic profiles of benzodiazepines that are metabolized primarily by conjugation, including temazepam and oxazepam, are not significantly altered in the elderly. These benzodiazepines may be more suitable choices for benzodiazepine therapy in elderly patients.

Renal disease can significantly affect benzodiazepine elimination. Because most parent benzodiazepines are highly protein-bound, glomerular filtration is low and their metabolism is less affected by renal disease. However, metabolites such as glucuronide conjugates can accumulate, because the kidney's ability to excrete these substances is compromised. Another consequence of renal disease is decreased plasma protein binding of benzodiazepines, which increases the concentrations of circulating free drug.

Conditions that cause hypoalbuminemia can increase the concentrations of free active drug. Hypoalbuminemia can occur as a result of liver disease (decreased synthesis of albumin), renal disease (extravascular protein loss), ascites and congestive heart failure (hemodilution), and severe burns (direct loss of albumin from the skin, a major site for albumin storage).

Individual Benzodiazepines

Alprazolam

Alprazolam is an intermediate-acting triazolobenzodiazepine that is primarily used to treat anxiety and depression. Its potency is about 20 times that of diazepam. A white powder with a pK_a of 2.4, alprazolam is soluble in methanol and ethanol and insoluble in water. Following oral administration, alprazolam is well absorbed, with a bioavailability of approximately 90%. Alprazolam is metabolized to α-hydroxyalprazolam and 4-hydroxyalprazolam by cytochrome P450 3A4. Both metabolites are less active than alprazolam and are typically detected in plasma at concentrations <10% of alprazolam concentrations. Almost all of a single dose of alprazolam is excreted within 72 h, with 80% excreted in urine and 7% in feces; twenty percent is excreted as unchanged alprazolam.

Clonazepam

Clonazepam is a long-acting benzodiazepine indicated for the treatment of seizure disorders and panic disorder. Clonazepam is a white to light yellow crystalline powder that is soluble in acetone, chloroform, and methanol. Following oral administration, clonazepam is well absorbed, with a bioavailability close to 100%. Clonazepam is primarily metabolized by reduction of the nitro group to form 7-aminoclonazepam, which is detected in plasma at concentrations similar to clonazepam. Cytochrome P450 3A4 mediates the formation of 7-aminoclonazepam. Up to 70% of a dose is eliminated in the urine over 7 days, mainly as 7-aminoclonazepam and 7-acetamidoclonazepam. Clonazepam is relatively unstable in postmortem specimens due to bacterial and thermal degradation.

Diazepam

Diazepam is a long-acting 1,4-benzodiazepine that is commonly prescribed for the management of anxiety, as an adjunct for the treatment of skeletal muscle spasm and status epilepticus, and as a minor tranquilizer or sedative. It is also used to reduce the effects of alcohol withdrawal. Diazepam is a white or yellow crystalline powder with a pK_a of 3.3 that is soluble in ethanol and chloroform and slightly soluble in water. Its oral bioavailability is about 100%. Following administration, diazepam is demethylated to form its primary active metabolite, nordiazepam, which accumulates in plasma with repeated administration. The CYP2C19 and CYP3A4 enzymes mediate the demethylation of diazepam; the CYP3A4 enzyme is involved in the formation of 3-hydroxy metabolites of diazepam, oxazepam, and temazepam. Following oral administration, much of a diazepam dose is eliminated in the urine as oxazepam glucuronide and conjugates of nordiazepam and temazepam.

Estazolam

Estazolam is an intermediate-acting triazolobenzodiazepine that is structurally related to both alprazolam and triazolam. It is prescribed orally in doses of 1–2 mg nightly for the treatment of insomnia, and its duration of action is approximately 6–8 hours. It is extensively metabolized in the liver to 4-hydroxyestazolam (by the CYP3A family of isoenzymes), followed by glucuronidation. Although the 4-hydroxy and 1-oxoestazolam metabolites have some pharmacological activity, their low potencies and abundance preclude significant contribution to the overall hypnotic effect of the drug.

Flurazepam

Flurazepam is a white crystalline hypnotic agent, soluble in chloroform, that is used to treat insomnia. Following oral administration, flurazepam is rapidly metabolized to N-1-desalkylflurazepam and N-1-hydroxyethylflurazepam, which may be responsible for much of flurazepam's observed effects. N-1-desalkylflurazepam accumulates in blood with repeated administration, achieving steady-state concentrations after 7–10 days of dosing. Steady-state N-1-desalkylflurazepam plasma concentrations are generally five to six times the concentrations observed following a single dose. Up to 60% of a flurazepam dose is eliminated in the urine within 48 h, and about 9% of a dose is eliminated in the feces. The primary urinary metabolite of flurazepam is conjugated N-1-hydroxyethylflurazepam.

Lorazepam

Lorazepam is an intermediate-acting benzodiazepine that is indicated for the treatment of anxiety. It is also used as a pre-anesthetic to alleviate anxiety, produce sedation, and decrease the ability to recall events related to a procedure. Following oral administration, lorazepam is well absorbed, with a bioavailability of 95% and a primary metabolic pathway of inactive glucuronide conjugate formation. Lorazepam glucuronide accumulates in plasma and attains concentrations greater than lorazepam. Approximately 75% of a lorazepam dose is eliminated over 5 days as lorazepam glucuronide in the urine; only a very small amount of lorazepam is eliminated as unchanged drug. Lorazepam can be difficult to detect, as most commercially available immunoassay screening tests do not have high cross-reactivity to this benzodiazepine.

Temazepam

Temazepam is a hypnotic agent indicated for the short-term treatment of insomnia. It is a white crystalline powder that is slightly soluble in water and freely soluble in methylene chloride. Temazepam is well absorbed following oral administration with a bioavailability close to 100%. The major metabolic pathway for temazepam is glucuronidation; smaller amounts of oxazepam and oxazepam glucuronide are formed. Approximately 80% of a dose is eliminated in the urine and 12% in the feces. Because the primary metabolic route is conjugation, temazepam pharmacokinetics are not significantly altered by changes in CYP3A4 activity.

Benzodiazepine Antagonist: Flumazenil

Flumazenil is an imidazobenzodiazepine derivative that acts as a competitive GABA antagonist in humans. The structure of flumazenil is illustrated in Fig. 20.5. Flumazenil does not antagonize the action of drugs binding to the GABA receptor at sites other than the benzodiazepine binding site (i.e., barbiturates, ethanol, and general anesthetics). Flumazenil can be used to reverse benzodiazepine effects such as sedation, respiratory depression, memory impairment, and psychomotor impairment. The duration and degree of antagonism are related to the dose administered and to the concentrations of flumazenil in plasma. Flumazenil is administered intravenously, and its actions are usually observed

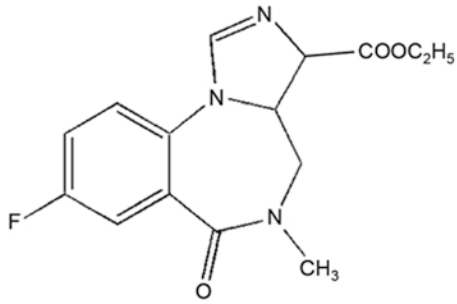


Fig. 20.5 Structure of flumazenil, a benzodiazepine antagonist

within minutes of administration. The complications associated with flumazenil administration are related to the reversal of benzodiazepine effects: flumazenil has been reported to precipitate withdrawal in patients who have been on benzodiazepine therapy long enough to develop tolerance and/or dependence. In addition, seizure activity can occur with flumazenil administration, especially if the patient has co-ingested a substance that causes seizure activity.

Flumazenil is not as highly protein-bound as most benzodiazepines, with only about 50% of the drug protein-bound. Following intravenous administration, flumazenil is rapidly distributed and eliminated; its half-life is 40 to 80 minutes. When flumazenil is administered as an antidote for benzodiazepine intoxication, the patient must still be monitored for signs of benzodiazepine intoxication, because the flumazenil may be eliminated more rapidly than the ingested benzodiazepine. Up to 95% of a dose of flumazenil is eliminated in the urine in 3 days and about 5–10% is eliminated in the feces. Flumazenil is excreted mainly as a desethyl carboxylic acid derivative and its glucuronide conjugate; less than 1% of unchanged drug is excreted in the urine.

Benzodiazepine Analogs

Illicit benzodiazepine use is common. As a consequence, clandestinely produced benzodiazepines have emerged, sometimes referred to as designer benzodiazepines. Many of these analogs involve variations of the 1,4-benzodiazepine skeleton based upon known structure-activity relationships, synthesis of active metabolites (e.g., desalkylflurazepam), as well as triazole, oxazole, pyridine, and thiophene analogs of these compounds. Although some were initially approved outside of the United States for medical use (e.g., phenazepam, etizolam, adinazolam), others are illicitly manufactured to circumvent drug legislation. Due to their relatively recent emergence, the pharmacological properties of some of these analogs are not yet well understood. However, their chemical classification (Table 20.3) provides some insight into their structure-activity relationships and relative potencies. The structures of some of these analogs are shown in Fig. 20.6 and selected drugs are discussed below.

Table 20.3 Chemical classification of selected benzodiazepine analogs

1,4-benzodiazepines
Desalkylflurazepam (norflurazepam)
Desmethylflunitrazepam (norflunitrazepam, fonazepam)
Diclazepam
Flubromazepam
Meclonazepam
Nifoxipam
Nimetazepam
Phenazepam
Triazolobenzodiazepines
Adinazolam
Bromazolam
Clonazolam
Flubromazolam
Flunitrazolam
Nitrazolam
Pyrazolam
Thioenotriazolodiazepines
Deschloroetizolam
Etizolam
Metizolam
Oxazolobenzodiazepines
Flutazolam

Etizolam

Etizolam is a thienotriazolodiazepine analog that is approved for clinical use outside of the United States as a sedative-hypnotic drug. Doses of

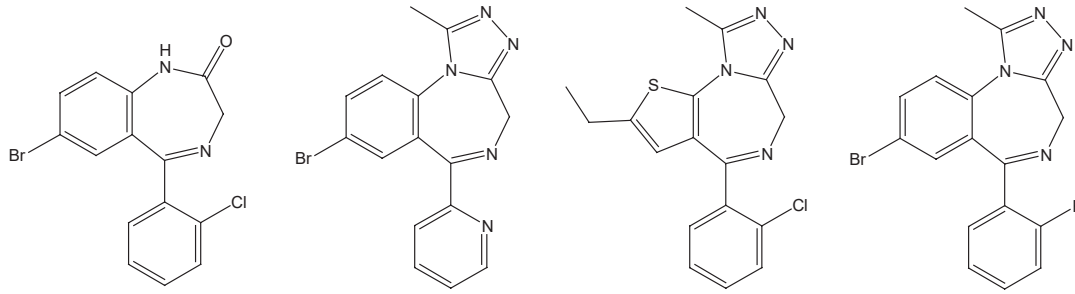


Fig. 20.6 Structures of selected benzodiazepine analogs

0.5–3 mg daily are administered orally. Single oral doses of 1 mg produced peak plasma concentrations of approximately 0.02 mg/L at 1 hour. The half-life is reported to be 7–15 hours and the drug is extensively metabolized by hydroxylation and glucuronidation. The major phase I metabolite (α-hydroxyetizolam) significantly contributes to the CNS-depressant effects of the drug, due to its approximately equipotent pharmacological activity and longer half-life.

Flubromazolam

Flubromazolam is a triazole analog of flubromazepam containing a fluorine atom, a bromine atom, and a methylated triazole moiety on the benzodiazepine backbone. It is a very potent benzodiazepine with only 0.25 mg needed to produce a sedative-hypnotic effect. It is metabolized primarily by hydroxylation and conjugation with glucuronic acid. Serum concentrations around 0.01 mg/L have been reported in forensic samples.

Phenazepam

Phenazepam is a benzodiazepine that was developed in the former Soviet Union in the 1970s and has been used in Russia to treat insomnia, anxiety, alcohol withdrawal, and seizures. It is not approved for use in the United States. It is a relatively potent benzodiazepine, and doses of 0.5 to 1.0 mg can produce significant central nervous system depression. After oral ingestion, the peak plasma concentration occurs at 4 hours. It has a longer half-life than most benzodiazepines, approximately 60 hours. It undergoes phase I metabolism by hydroxylation to produce 3-hydroxyphenazepam which is also psychoactive. The metabolite then undergoes phase II metabolism and is excreted as a glucuronide. Two other metabolites have also been reported, 5-bromo-(2-chlorophenyl)-2-aminobenzophenone (ABPH) and 6-bromo-(2-chlorophenyl) quinazoline-2-one.

Pyrazolam

In 2012, another designer benzodiazepine, pyrazolam, began appearing on the illicit drug market. Pyrazolam is structurally similar to alprazolam, with pyrazolam having a bromine atom on the benzene ring of the benzodiazepine nucleus instead of chlorine and a pyridine ring instead of a benzene ring on C-6 of the diazepine ring. The presence of the strong electron-withdrawing groups again leads to the potency of pyrazolam and small doses prescribed. Ingestion of 1 mg produced a peak blood concentration of around 0.05 mg/L. Surprisingly, no expected hydroxylation, dealkylation, or dehalogenation phase I or phase II metabolites have been identified.

Analysis

Many difficulties are associated with the attempt to take a comprehensive approach to benzodiazepine analysis. A large number of benzodiazepines with different functional groups exist on

the benzodiazepine nucleus. Many metabolites of benzodiazepines are pharmacologically active and should be quantified to assess the overall effects of benzodiazepines in a particular case. Benzodiazepine potencies may vary by several orders of magnitude, so analytical methodologies need different detection limits to identify therapeutic use. Low-dose benzodiazepines (e.g., triazolam) may be difficult to detect without specialized instrumentation. With many benzodiazepines, establishing a simultaneous method for blood and urine specimen analysis is difficult, because the target compound is often different. Moreover, urinary benzodiazepine products are conjugated and a hydrolysis step is required to improve detectability.

There are many commercially available screening tests for benzodiazepines. Some are designed to test urine, while others test a wide range of matrices, making them applicable to postmortem toxicology. Often the target analyte is oxazepam or nordiazepam, and good cross-reactivity to a number of benzodiazepines can be achieved. More recently, specific assays for flunitrazepam have been developed. Typically, no specimen pretreatment is necessary; however, sensitivity of these assays may be enhanced by an enzymatic hydrolysis step, because the predominant urinary products are conjugated species. One limitation to the use of immunoassays is that certain benzodiazepines, such as lorazepam, do not have sufficient cross-reactivity with the assay antibody to identify that benzodiazepine's therapeutic use.

Benzodiazepines can be separated from biological specimens by liquid-liquid extraction or by solid-phase extraction. When analyzing urine specimens, hydrolysis is necessary to cleave the glucuronide conjugate. Enzymatic hydrolysis is preferred over acid hydrolysis; some benzodiazepines are unstable in acid and rearrange to form benzophenones. Adjusting the pH to 9–10 allows extraction of benzodiazepines into an immiscible organic solvent. Solid-phase extraction procedures also rely on pH adjustment, with the final pH dependent on the type of solid phase being used. After application of the pH-adjusted specimen to the column, buffers and solvents are used to wash the column of endogenous substances or other drugs. This is followed by solvent elution of the benzodiazepines from the column.

Chromatographic separation of benzodiazepines can be accomplished using either gas or liquid chromatography. Gas chromatography (GC) can analyze many benzodiazepines without derivatization, among them chlordiazepoxide, diazepam, nordiazepam, flurazepam, and alprazolam. Disadvantages to using GC analysis of benzodiazepines include the thermal instability of chlordiazepoxide, which can degrade during operating temperatures. The most significant drawback to using GC is that some of the more polar drugs within the class have poor chromatographic properties. Some of the diazolobenzodiazepines and triazolobenzodiazepines require high temperatures for elution from a GC column; these compounds are also very sensitive to chromatographic conditions, and less than optimal results may be obtained if regular instrument maintenance is not performed. Drugs with a hydroxyl group (such as oxazepam, temazepam, and lorazepam) or a nitro group (such as clonazepam and nitrazepam) can display poor chromatographic characteristics. Derivatization by gas chromatography is often necessary for the analysis of these compounds. Because of this requirement, liquid chromatography is the preferred chromatographic separation technique to detect individual benzodiazepines and their metabolites. A reverse-phase C_8 or C_{18} column is commonly used to provide analytical separation. Isocratic mobile phases can provide adequate separation for most applications. After chromatographic separation, mass spectrometry (MS) is used to provide definitive identification of the benzodiazepines.

Analytical sensitivity is another consideration. Low-dose benzodiazepines may require specialized analytical approaches, such as GC-MS with chemical ionization (CI). The electrophilic halogen present can be exploited for this purpose when negative CI is utilized. However, not all laboratories have CI capability with GC-MS. As a result, targeted LC-MS-based approaches (e.g., LC-MS/MS) provide sufficient sensitivity for single-dose or therapeutic drug concentrations.

Interpretation

Therapeutic ranges for benzodiazepines reflect their differences in potency. Many drugs, like diazepam and chlordiazepoxide, have therapeutic concentrations around 2 mg/L. Others, like alprazolam and lorazepam, have therapeutic concentrations in the 0.05–0.1 mg/L range. In contrast, low-dose benzodiazepines (e.g., triazolam) may be present at low ng/mL concentrations. Active metabolites are also a factor to be considered when evaluating the amount of active benzodiazepine in the blood.

Although the presence of benzodiazepines is a relatively common finding in postmortem cases, few intoxication cases due exclusively to benzodiazepines have been reported due to their high therapeutic indices. However, there have been some studies that suggest alprazolam is relatively more toxic in overdose situations than other benzodiazepines. Generally, benzodiazepines are involved in drug deaths as a result of being combined with alcohol or other drugs, with the cause of death attributable to alcohol and drug or multiple drug intoxication.

One complication in the interpretation of postmortem benzodiazepine concentrations is that some display in vitro instability. For instance, chlordiazepoxide is broken down in the blood to demoxepam and nordiazepam. Drugs with a nitro group, such as clonazepam and nitrazepam, can be reduced in vitro to their respective amino products. Chlordiazepoxide standards are unstable in water or methanol and should be prepared fresh or in aprotic solvents such as acetonitrile. Stability issues must also be considered during specimen storage and transport.

The instability of the nitrobenzodiazepines should also be considered for some of the newer nitro-analogs (e.g., clonazolam, diclazepam, flunitrazolam, fonazepam, nifoxipam, nimetazepam, nitrazolam, meclonazepam).

A negative result of a benzodiazepine screen must be interpreted in the context of the methodology used. The discussion of benzodiazepine analysis in the previous section clearly indicated that different methodologies have different abilities to identify certain benzodiazepines. If the case history indicates the presence or involvement of a particular benzodiazepine, then a method must be selected that would identify the drug or metabolite of interest. This is of particular importance when benzodiazepines are implicated in drug-facilitated sexual assault, where a single dosage may be administered, and significant delays between the time of the alleged incident and specimen collection may exist.

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