SL651498, a GABA$_A$ Receptor Agonist with Subtype-Selective Efficacy, as a Potential Treatment for Generalized Anxiety Disorder and Muscle Spasms

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ABSTRACT

SL651498 (6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2, 9-dihydro-1*H*-pyrido[3,4-*b*]indol-1-one) was identified as a drug development candidate from a research program designed to discover subtype-selective GABA$_A$ receptor agonists for the treatment of generalized anxiety disorder and muscle spasms. The drug displays high affinity for rat native GABA$_A$ receptors containing α_1 (K_i = 6.8 nM) and α_2 (K_i = 12.3 nM) subunits, and weaker affinity for α_5-containing GABA$_A$ receptors (K_i = 117 nM). Studies on recombinant rat GABA$_A$ receptors confirm these findings and indicate intermediate affinity for the $\alpha_3\beta_2\gamma_2$ subtype. SL651498 behaves as a full agonist at recombinant rat GABA$_A$ receptors containing α_2 and α_3 subunits, and as a partial agonist at recombinant GABA$_A$ receptors expressing α_1 and α_5 subunits. SL651498 produced anxiolytic-like and skeletal muscle relaxant effects qualitatively similar to those of benzodiazepines (BZs) [minimal effective dose (MED): 1 to 10 mg/kg, i.p. and 3 to 10 mg/kg, p.o.]. However, unlike these latter drugs, SL651498 induced muscle weakness, ataxia or sedation at doses much higher than those having anxiolytic-like activity (MED: 30 to 100 mg/kg, i.p. or p.o.). Moreover, in contrast to BZs, SL651498 did not produce tolerance to its anticonvulsant activity or physical dependence. It was much less active than BZs in potentiating the depressant effects of ethanol or impairing cognitive processes in rodents. The differential

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profile of SL651498 as compared to BZs may be related to its selective efficacy at the α_2- and α_3-containing GABA$_A$ receptors. This suggests that selectively targeting GABA$_A$ receptor subtypes can lead to drugs with increased clinical specificity. SL651498 represents a promising alternative to agents currently used for the treatment of anxiety disorders and muscle spasms without the major side effects seen with classical BZs.

INTRODUCTION

Introduced over 40 years ago, benzodiazepines (BZs) quickly became the most widely used psychotropic drugs. Their marked anxiolytic, hypnotic, anticonvulsant and muscle relaxant properties and their relatively good safety margin, rapidly elevated BZs to the treatment of choice for common and recurrent conditions such as anxiety states, muscle tension and insomnia. However, in recent years, attitudes toward these compounds have greatly changed, and growing awareness and concern about dependence liability, withdrawal phenomena, and short and long-term side effects have brought the long-term use of these compounds into question (26,61). BZs produce their pharmacological effects through positive allosteric modulation of the action of GABA at ionotropic GABA$_A$ receptors (1,5,38). GABA$_A$ receptors have a pentameric structure formed by the assembly of subunits from different families which possess genetic variants (α_{1-6}, β_{1-4}, γ_{1-3}, ρ_{1-3}, ε_1, π_1, and δ_1). The existence of at least 16 distinct subunits leads to a substantial GABA$_A$ receptor heterogeneity. The most abundant GABA$_A$ receptor subtypes contain at least one member of the α, β, and γ subunit classes. Sensitivity to BZs is conferred by the γ_2 subunit as well as by adjacent α_1, α_2, α_3, and α_5 subunits.

The search has begun for compounds chemically unrelated to BZs with more specific therapeutic actions and without concomitant unwanted effects. This has led to the development of drugs that selectively bind to specific GABA$_A$ receptor subtypes such as the hypnoselective agent, zolpidem, which preferentially recognizes the α_1-containing GABA$_A$ receptor (11) and/or show different efficacies at GABA$_A$ receptors (e.g., bretazenil, imidazenil, Y-23684) (15,33,62). The heterogeneity of GABA$_A$ receptors has prompted speculation that a particular behavioral response might be associated with an action at a defined receptor subtype. This idea is now largely substantiated by several findings using either GABA$_A$ receptor subtype-selective ligands or mutant mice in which a specific GABA$_A$ receptor subunit is inactivated. For example, zolpidem was found to decrease exploration or induce sleep at doses much lower than those inducing myorelaxation, while non selective GABA$_A$ receptor full agonists affect primarily muscle strength. This suggests that the hypnotic but not the myorelaxant activity is related to an interaction with α_1-containing GABA$_A$ receptors (20,43). In line with these findings are studies using mice with point-mutated zolpidem- or diazepam-insensitive GABA$_A$ receptor subtypes. These studies showed that the sedative actions of zolpidem and diazepam were absent in α_1 knock-in mice, whereas the muscle relaxation induced by the administration of diazepam was still present in these animals (9,31,49,56). Moreover, preferential GABA$_A$ α_1 subtype full agonists are generally found to display weaker anxiolytic-like activity in animals than non-selective agents (e.g., 20,50). In addition, the anxiolytic-like action of the non-selective GABA$_A$ receptor agonist diazepam is absent in α_2 but not in α_1 or α_3 knock-in mice. This suggests a major role for the α_2-containing GABA$_A$ receptor in the anxio-
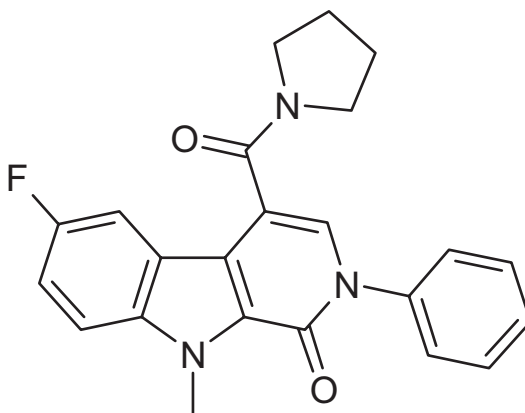
Fig. 1. Chemical structure of SL651498 {(6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1*H*-pyrido[3,4-*b*]indol-1-one}.

lytic activity of BZs (49). However, a role for the GABA α_1 subtype in the anxiolytic effects of BZs cannot be totally ruled out as several studies demonstrated that the preferential GABA$_A$ α_1 subtype antagonist β-CCT completely blocked anxiolytic-like effects of BZs in rodents (2,17,48). Together, these findings suggest that the anxiolytic effects of GABA$_A$ receptor agonists are mediated by the α_2 and, to a lesser extent, the α_1 GABA$_A$ receptors. These findings also suggest that the sedative/hypnotic and muscle-relaxant effects of GABA$_A$ receptor agonists involve primarily the α_1 and the α_3 and/or α_5 GABA$_A$ receptors, respectively. Here we report on the preclinical pharmacological profile of SL651498, a pyridoindole derivative which has been selected on the basis of its functionally selective agonist activity at the α_2 and α_3 GABA$_A$ receptor subtypes

CHEMISTRY

SL651498, 6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2, 9-dihydro-1*H*-pyrido[3,4-*b*]indol-1-one, is a novel pyridoindole derivative (Fig. 1) (13) It belongs to the 1*H*-pyrido[3,4-*b*]indole-4-carboxamide chemical series that possesses anxiolytic-like properties. SL651498 has a low molecular weight (389.4 g/mol), a melting point of 202 –205°C and a log *D* of 3.18 (pH = 7.4; octanol–water/liquid chromatographic method). The physicochemical properties of the compound thus fall within the "Lipinski" rule of 5 (28). The compound is a white crystalline powder that is insoluble in water. It is stable to heat, daylight and humidity. SL651498 is prepared in a seven-step synthesis from 4-fluorophenylhydrazine (I) (Fig. 2). Thus, the hydrazone (II), prepared by reaction of the hydrazine (I) with 2-ketoglutaric acid in the presence of base, is cyclized and esterified in a second step under acidic conditions to produce an indole diester (III). This product is *N*-methylated (IV) and condensed with the dimethylformamide dimethylacetal affording an enaminodiester (V). The pyrido[3,4-*b*]indole heterocycle (VI) is formed in the next step by reacting the latter product with aniline (addition-elimination followed by cyclization). After hydrolysis of the ester function of the cyclized product, amidification of the resulting acid (VII) with pyrrolidine under standard conditions affords SL651498.
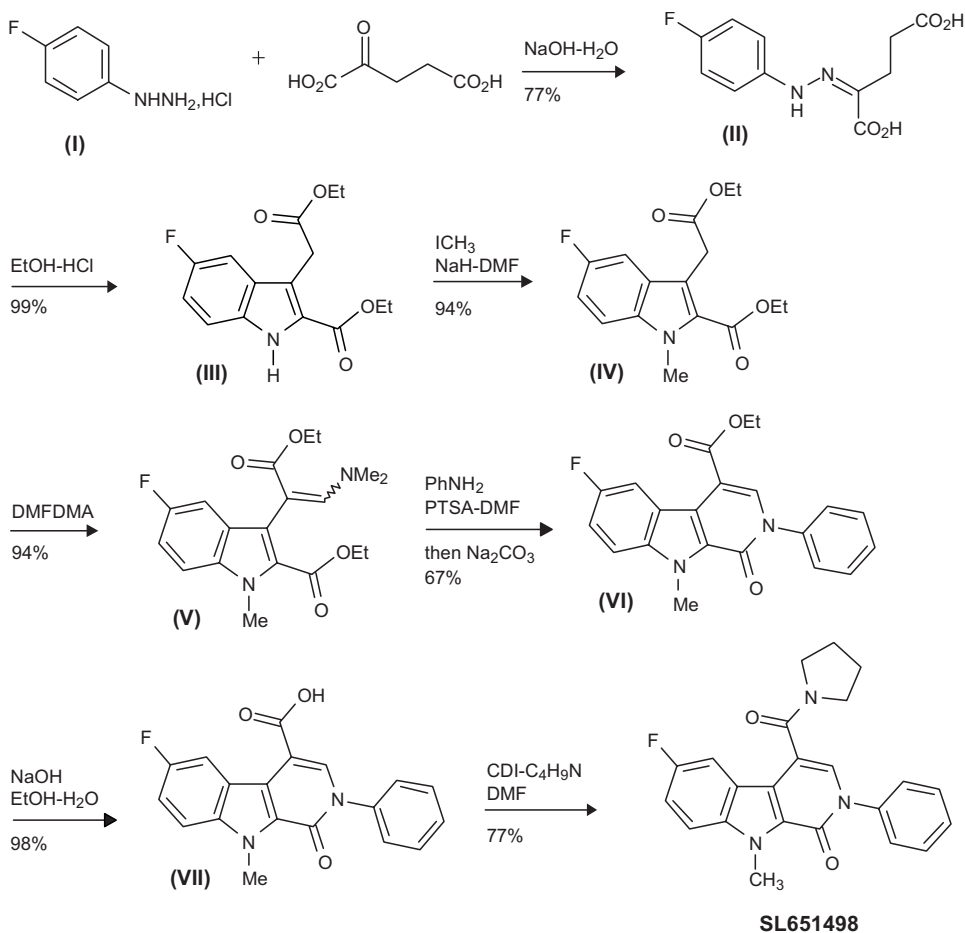
Fig. 2. Synthesis of SL651498.

PHARMACOLOGY

Receptor Binding Studies

Studies of [³H]flumazenil binding to native rat $GABA_A$ receptor subtypes showed that SL651498 was more potent at displacing [³H]flumazenil binding to membranes from cerebellum ($K_i = 6.8$ nM) and spinal cord ($K_i = 12.3$ nM) than from hippocampus ($K_i = 117$ nM). Cerebellum and spinal cord are two brain areas enriched in $GABA_A$ α_1 and α_2 subtypes, respectively, while hippocampus is a brain area containing $GABA_A$ α_5 receptors. In contrast, the classical BZ diazepam displaced [³H]flumazenil binding with similar affinity in membranes from cerebellum, spinal cord or hippocampus (Table 1) (19). Essentially similar results were obtained when studying the recombinant rat $GABA_A$ receptor subtypes (Table 1). In addition, SL651498 showed no displacement in more than 90 binding assays for various neurotransmitters, peptides and hormones, demonstrating its specificity for the $GABA_A$ receptor.

TABLE 1. *Effects of SL651498 on the binding of [³H]flumazenil to recombinant rat GABA$_A$*
receptors, and to native rat GABA$_A$ receptors in the cerebellum, the spinal cord
and the hippocampus, which predominantly express α$_1$, α$_2$ and α$_5$ subtypes, respectively

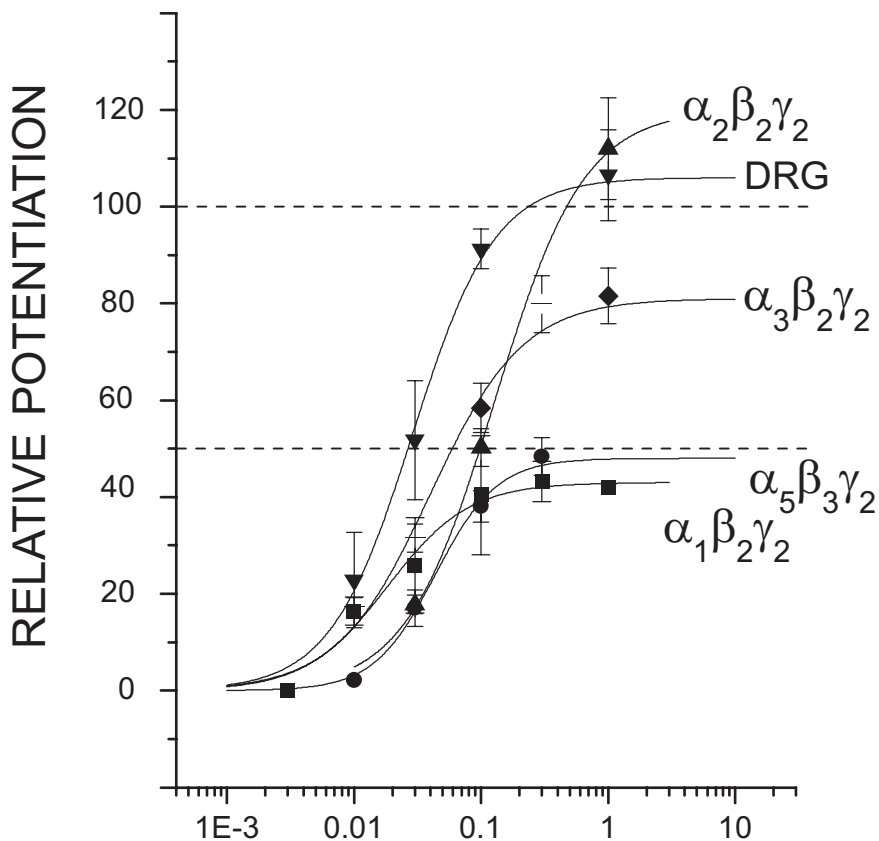|	GABA$_A$ receptor subtype K_i (nM)			
Native	α$_1$	α$_2$		α$_5$
SL651498	6.8 ± 1.9	12.3 ± 1.9		117 ± 13[a]
Diazepam	5.8 ± 1.9	4.8 ± 1.9		13.4 ± 4.7[a]
Recombinant	α$_1$β$_2$γ$_2$	α$_2$β$_2$γ$_2$	α$_3$β$_2$γ$_2$	α$_5$β$_3$γ$_2$
SL651498	17.0 ± 1.5	73.0 ± 7.0	80.3 ± 10.4	215 ± 7
Diazepam	14.0 ± 2.1	7.8 ± 1.1	13.9 ± 0.5	9.8 ± 1.1

[a] Studied in the presence of 5 μM zolpidem in order to mask the GABA$_A$ α$_1$ and α$_2$ subtypes. Data represent the mean ± S.E.M. of at least 3 experiments performed in duplicate.

Efficacy at GABA$_A$ Receptor Subtypes

Rational targeting of specific GABA$_A$ receptor subtypes can be achieved via preferential affinity for a particular receptor subtype and/or by receptor subtype-selective efficacy (46). The efficacy of GABA$_A$ receptor ligands can be assessed *in vitro* by studying the potentiation of chloride currents induced by rapid application of GABA in transfected cells expressing different combinations of GABA$_A$ receptors. Classical BZs, such as diazepam have high efficacy at nearly all receptor subtypes (full agonists). In contrast, partial agonists, as typified by bretazenil or imidazenil have reduced efficacy compared to diazepam at all receptors (21,46,60). Analysis of the concentration-dependence of the potentiation by SL651498 of the chloride current induced by 0.3 μM GABA in rat α$_1$β$_2$γ$_2$ cell lines showed that the maximal potentiation produced by SL651498 represents about half of that of the full agonist zolpidem. While at GABA$_A$ receptors expressing rat α$_2$β$_2$γ$_2$ and α$_3$β$_2$γ$_2$ subunits, the efficacy of SL651498 was comparable to that of diazepam, it was much lower than that of the BZ in rat α$_5$β$_3$γ$_2$ cell lines (maximal potentiation about 50% of that of diazepam) (Figs. 3 and 4AB) (19). Moreover, on cultured rat dorsal root ganglia (DRG) cells which are enriched in native α$_2$ subunits, SL651498 displayed a high intrinsic activity similar to that of diazepam (Fig. 3) (19). Altogether, these data indicate that SL651498 behaves as a full agonist at α$_2$- and α$_3$-containing GABA$_A$ receptors, and as a partial agonist at α$_1$- and α$_5$-containing GABA$_A$ receptors.

In vivo, the efficacy of GABA$_A$ receptor ligands can be assessed by studying their ability to modify the latency to clonic seizures produced by isoniazid. Isoniazid inhibits glutamic acid decarboxylase, the enzyme that catalyzes the synthesis of GABA from glutamic acid, thereby reducing the neuronal stores of GABA available for nerve impulse-mediated release of this transmitter (30). The maximal delay in onset of isoniazid-induced seizures produced by a test compound may, therefore, be taken as an index of increased GABAergic function. This index has been proposed as an *in vivo* measure of the intrinsic activity of GABA$_A$ receptor ligands (32). Diazepam produced a larger increase in this measure than bretazenil, which is consistent with the well-acknowledged idea that diazepam shows higher intrinsic activity than bretazenil. The GABA$_A$ α$_1$ subtype agonist zolpidem produced a very large increase in the latency to clonic seizures produced by iso-

Fig. 3. GABA-induced chloride currents potentiation by SL651498 relative to zolpidem ($\blacksquare$, $\alpha_1\beta_2\gamma_2$) or diaze-pam ($\blacktriangle$, $\alpha_2\beta_2\gamma_2$; $\blacklozenge$, $\alpha_3\beta_2\gamma_2$; $\bullet$, $\alpha_5\beta_3\gamma_2$) in HEK293 cells stably expressing rat recombinant GABA$_A$ receptor sub-types, and rat dorsal root ganglion (DRG) neurons in culture which contain exclusively native α_2-containing GABA$_A$ receptors ($\blacktriangledown$).
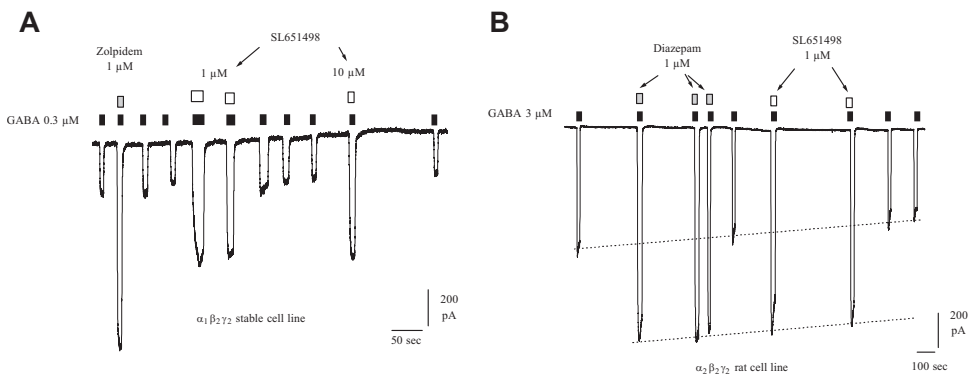
Fig. 4. GABA-induced chloride currents potentiation by SL651498 in two HEK293 cells stably expressing rat recombinant (A) $\alpha_1\beta_2\gamma_2$ and (B) $\alpha_2\beta_2\gamma_2$ receptor subtypes, respectively. Comparison with zolpidem or diazepam.
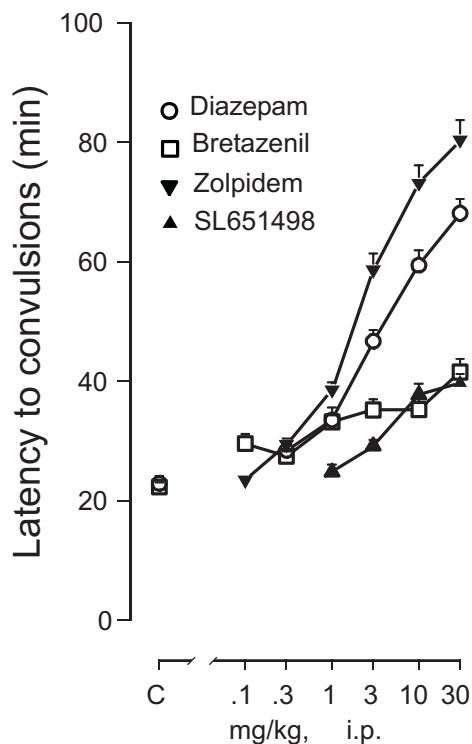
Fig. 5. Evaluation of the intrinsic efficacy of SL651498, diazepam, bretazenil and zolpidem at the GABA$_A$ α_1 subtype by measuring their anticonvulsant activity against clonic seizures produced in mice by 800 mg/kg s.c. of isoniazid. Data of bretazenil and zolpidem are from refs. 18,20.

niazid, greater than that seen with diazepam (53). The finding that SL651498 displayed similar low efficacy as bretazenil (Fig. 5), together with the finding that the former antagonized the anti-isoniazid effects of diazepam and zolpidem (19) strengthens the electrophysiological data showing that SL651498 behaves *in vivo* as a partial agonist at GABA$_A$ receptor subtypes expressing α_1 subunits.

Discriminative Stimulus Effects

With GABA$_A$ receptor ligands, drug discrimination procedures provide additional information that assists in identifying receptor subtypes involved in the actions of these drugs. The stimulus effects of BZs has been analyzed in great detail using chlordiazepoxide (7) and diazepam (22), or more recently, zolpidem (52). In general, there is a complete cross substitution between different BZs. In contrast, selective GABA$_A$ α_1 subtype agonists, such as zolpidem, produce only partial substitution for the BZ cue, suggesting that this latter stimulus is mediated by GABA$_A$ receptor subtypes other than the α_1-containing ones. The interoceptive stimulus produced by SL651498 was found to substitute completely for the chlordiazepoxide cue, whereas it produced only partial substitution for the zolpidem α_1-dependent cue (8, 52). This suggests that GABA$_A$ receptor subtypes other than those bearing the α_1 subunit are involved primarily in these effects (19).

THERAPEUTIC INDICATIONS

Anxiolytic Activity

The anxiolytic-like properties of SL651498 were examined using a variety of rodent models. These included conflict procedures [punished lever pressing (50), punished drinking (58), and four–plate (4) tests], exploration models [elevated plus-maze (42) and light/dark (36) tests], a fear/anxiety defense test battery (16). Models also included several test procedures based on stress-induced changes in physiological [isolation- or cat odor-induced hyperthermia (3,27)] or behavioral [fear-potentiated startle (10) and cat exposure (3) tests] parameters. Conflict procedures, exploration models, and the fear-potentiated startle and defense test battery have been extensively and successfully used for the screening of BZs, suggesting that they may model certain aspects of generalized anxiety disorder. However, tests based on unavoidable exposure to predator stimuli (e.g., cat) are much less sensitive to the action of BZs. This indicates that they may model aspects of other anxiety disorders. In line with this idea, diazepam (1 to 10 mg/kg) elicited marked anxiolytic-like effects in all conflict paradigms, in exploration tests, in the fear-potentiated startle procedure and in the defense test battery. Moreover, the drug counteracted the increase in body temperature following isolation stress. In contrast, diazepam was inactive in those situations where the stress stimulus was a cat or a cat odor (Fig. 6 and Table 2).

In anxiety tests SL651498 displayed a profile qualitatively similar to diazepam. It produced clear-cut anxiolytic-like activity at either 1 to 10 mg/kg i.p. or 3 to 10 mg/kg p.o. in all BZ-sensitive procedures, and failed to produce behavioral or physiological changes after cat or cat-odor exposure. It is important to note that SL651498, at the doses tested in anxiety tests, did not modify unpunished responding in conflict procedures or spontaneous locomotor activity in exploration models or in the defense test battery. Clearly, these findings have a direct bearing on the issue of the behavioral selectivity of any changes observed in anxiety-related responding. The analysis of the duration of the anxiolytic-like action of SL651498 (10 mg/kg, p.o.) indicated that the effects lasted for more than 3 h in

TABLE 2. *Effects of SL651498 and diazepam in rodent models of anxiety*

Tests	MED (mg/kg), i.p. (p.o.)	
	SL651498	Diazepam
Drinking conflict test in rats	(10)	(10)
Elevated plus-maze in rats	(3)	(20)
Elevated plus-maze in mice	10	3
Light/dark test in mice	10	2
Four-plate test in mice	3	2
Mouse defense test battery: Flight	3 (10)	3 (3)
Mouse defense test battery: Risk assessment	10	1
Mouse defense test battery: Defensive aggression	10	1
Mouse defense test battery: Contextual anxiety	10	>3
Cat exposure test in rats	>3	>3
Cat odor-induced hyperthermia in rats	(>30)	(>10)

MED, minimal effective dose.
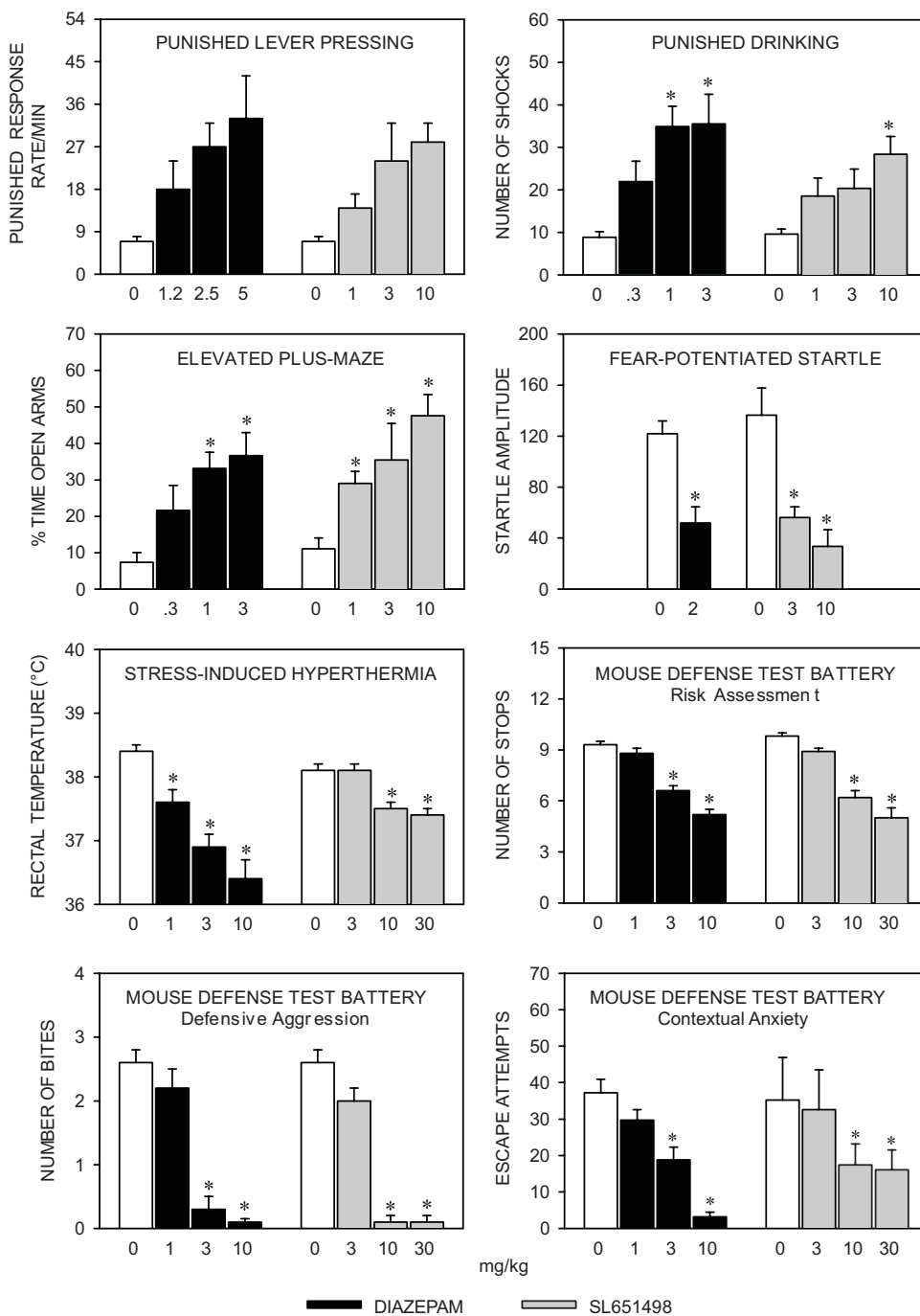
Fig. 6. Comparison of the effects of SL651498 and diazepam in several rat (punished lever pressing, punished drinking, elevated plus-maze and fear-potentiated startle tests) and mouse (stress-induced hyperthermia and defense test battery) models of anxiety following intraperitoneal and oral admistrations, respectively. *$P < 0.05$.

the elevated plus-maze test in rats (19). Moreover, after repeated administration for 13 days, the effects of SL651498 (1 to 10 mg/kg, p.o., twice a day) in the defense test battery were still evident, indicating that no tolerance to the anxiolytic-like activity had developed. Indeed, repeated administration of SL651498 tended to potentiate its effects as was evidenced by the lower minimal effective dose following this treatment (i.e., 3 vs. 10 mg/kg after acute treatment) on some measures (19).

The finding that the anxiolytic-like activity of SL651498 was antagonized by the non-selective $GABA_A$ receptor antagonist flumazenil indicates that central GABA/BZ receptors are involved in these effects. The weak affinity of SL651498 at the $GABA_A$ receptor α_5 subtype would suggest that this receptor subtype is not responsible for the anxiolytic-like action of the drug. The possibility that the $GABA_A$ receptor α_1 subtype may be involved in the anxiolytic-like effects of SL651498 was investigated by testing the effects of a co-administration of SL651498 and the selective $GABA_A$ receptor α_1 subtype antagonist, β-CCT*, in several anxiety tests. Results showed that β-CCT did not modify the anxiolytic-like action of SL651498 in several tests (e.g., the elevated plus-maze, the four-plate test, the isolation-induced hyperthermia procedure, and the defense test battery). These results are consistent with the hypothesis that anxiolysis produced by SL651498 involves $GABA_A$ receptor subtypes other than those bearing the α_1 subunit, but whether α_2 and/or α_3-containing $GABA_A$ receptors mediate this effect remains to be investigated.

Muscle Relaxant Activity

The functional selectivity of SL651498 for α_2 and α_3 $GABA_A$ receptor subtypes in the spinal cord (14), where they have been suggested to play an important role in the muscle relaxant effects of benzodiazepines (37), prompted us to investigate the effects of SL651498 in a variety of animal models predictive of muscle relaxant activity (Table 3). Results showed that the drug reduces the polysynaptic reflex (Aβ–Aδ fiber mediated reflexes) (54) in normal rats after both acute (3 to 10 mg/kg, i.p., and 10 to 30 mg/kg, p.o.) and repeated administration (10 mg/kg, i.p., daily for 8 days). The finding that the drug was still active in rats with spinal lesions suggests that the inhibition of the polysynaptic reflex in normal rats was due, at least in part, to a depressant action at the spinal level. By contrast, SL651498 was inactive on the monosynaptic reflex in normal rats, indicating an inhibitory action at the spinal level. SL651498 (3 mg/kg, i.v.) also inhibited C fiber evoked reflexes in anesthetized rats, indicating an analgesic effect. The drug also affected the stretch reflex in spastic rats (Ia–II fiber evoked reflexes; 10 to 30 mg/kg, i.p.). Rats treated with the inflammation-inducing agent (Complete Freund's Adjuvant containing *Mycobacterium butyricum*) is a model more closely related to human pain states in which muscle tone is exacerbated (41). In this model the inhibition of polysynaptic reflexes by SL651498 (3 mg/kg, i.p.) was greater when compared to the effect observed in normal rats. Overall, the efficacy and potency of SL651498 in these models is comparable to that of several reference compounds, including diazepam, and the prototypical muscle relaxant agents, tetrazepam, baclofen and tizanidine. However, unlike these compounds, SL651498 showed muscle relaxant effects at non-sedative doses. These data suggest a potential for this drug in the treatment of pathological conditions (such as muscle spasms) associated with musculoskeletal disorders. SL651498 may also be effective in the

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* β-CCT is β-carboline-3 carboxylate-t-butyl ester.

treatment of spasticity due to primary neurological diseases in which an exaggerated muscle tone is of pathophysiological importance.

SIDE EFFECT PROFILE

Central Depressant Effects

Central depressant effects of traditional BZs generally seen as ataxia, muscle weakness and sedation are usually manifested at doses only slightly higher than those producing anxiolytic-like action. For example, diazepam impaired the performance of rodents in the rotarod (12) and the grip strength (35) tests, and increased the total "power" in the EEG (three models generally used to examine the ataxic, muscle-relaxant and sedative properties of psychoactive drugs, respectively) at doses ranging from 1 to 10 mg/kg, i.p.. This clearly overlaps with the dose range that produces anxiolytic-like activity (1 to 3 mg/kg, i.p.) (Tables 2 and 4 and Fig. 7). In contrast, SL651498 produced motor impairment and

TABLE 3. Summary of the effects of SL651498 on spinal reflexes evoked in rats.
Comparison with diazepam, tetrazepam, baclofen, and tizanidine

Drugs	Dose (mg/kg)	Flexor reflex			Stretch reflex	H reflex[b]	Nociceptive reflex
		Normal rats	Rats with spinal lesion	CFA-treated rats[a]	Spastic rats	Normal rats	Anesthetized rats
SL651498	3 i.p.	–39*		–62**			
	10 i.p.	–67**	–30**		–29**	–14 ns	
	30 i.p.				–36**		
	10 p.o.	–35**	–29**				
	30 p.o.	–58**	–40**				
	3 i.v.						–43**
Diazepam	1 i.p.	–51**			–31**		
	3 i.p.	–67**	–37**	–25**	–37**	3 ns	
Tetrazepam	3 i.p.			–46**			
	10 i.p.	–56**	–47**	–54**	–29*	–11 ns	
	15 i.p.	–55**			–58**	–8 ns	
	3 i.v.						–26**
Baclofen	3 i.p.	–67**	–50**	–65**	–42**	–33**	
	10 i.p.	–80**			–69**	–57**	
Tizanidine	3 i.p.	–68**	+133*	–80*	–52**	–24 ns	
	10 i.p.	–91**			–50*	–62 **	

Values indicate maximal inhibition, expressed as percentage change of baselines, during the first hour after the administration of the compounds. *$P < 0.05$; **$P < 0.01$ (vs. baseline levels).
[a] Rats were treated with the inflammatory-inducing agent Complete Freund's Adjuvant containing *Mycobacterium butyricum* (54);
[b] The H (Hoffmann) reflex from the small muscles of the foot was observed upon stimulation of the sciatic nerve.

sedation at doses higher than those producing anxiolytic-like effects. For example, the drug induced slight sedative and ataxic effects at doses (30 to 100 mg/kg, i.p.) which are 10 to 30 times higher than those producing anxiolytic-like activity in the different models (1 to 10 mg/kg, i.p.). Altogether, the results from activity tests showed that at doses lower than 30 mg/kg i.p. or p.o. SL651498 did not significantly modify the performance of animals, while anxiolytic-like activity appeared at doses ranging between 1 to 10 mg/kg, depending on the test. We can, therefore, anticipate a higher therapeutic ratio for SL651498 compared to classical BZs in future clinical studies. While the low intrinsic activity of SL651498 at the $GABA_A$ α_1 subtype may account for its limited propensity to produce sedation, the weak affinity and efficacy of the compound at the α_5-containing $GABA_A$ receptor subtype may account, at least in part, for its profile in the grip strength and loaded grid tests. The finding that the $GABA_A$ α_1 subtype antagonist β-CCT did not block the effects of SL651498 in the rotarod test indicates that the ataxic effects observed at doses higher than 30 mg/kg are not primarily mediated by the α_1 subtype. This finding is in agreement with the observation that mice carrying the H101R point mutation in the α_1 subunit still displayed ataxia following the administration of diazepam (34).

Cognitive Effects

Anterograde amnesia is one of the troublesome adverse effects of the BZs, especially when they are used as tranquillizers. In animal procedures, BZs can induce disturbances in learning (6,25,55). For example, the Morris water maze task in rats (39) and the T-maze in mice (57) have been extensively used for studying the effects of BZs on spatial memory. These studies showed that administration of a BZ before the first trial produces a response deficit in the following trials, indicating a failure of acquisition. SL651498 was tested in

TABLE 4. Summary of the side-effect profile of SL651498 and diazepam in animal studies

| Tests	MED (mg/kg), i.p. (p.o.)	
	SL651498	Diazepam
EEG in rats	30 (30)	1
Rotarod in mice	100 (100)	3
Rotarod in rats	30 (60)	10
Grip strength in mice	60 (>100)	3 (3)
Grip strength in rats	30 (>100)	3 (30)
Loaded grid in mice	30	3
Loaded grid in rats	60 (>100)	3
Mouse defense test battery: line crossings	(>30)	(10)
Horizontal wire test in mice	(>30)	(1)
Morris water maze in rats	>10	10
T-maze in mice	>10	
Passive-avoidance in mice	10	(1)
Object recognition task in mice	(>30)	(1)
Interaction with alcohol in mice	(30)	(1)
Tolerance to anticonvulsant activity in mice	>30	5
Physiological dependence in mice	>30	5
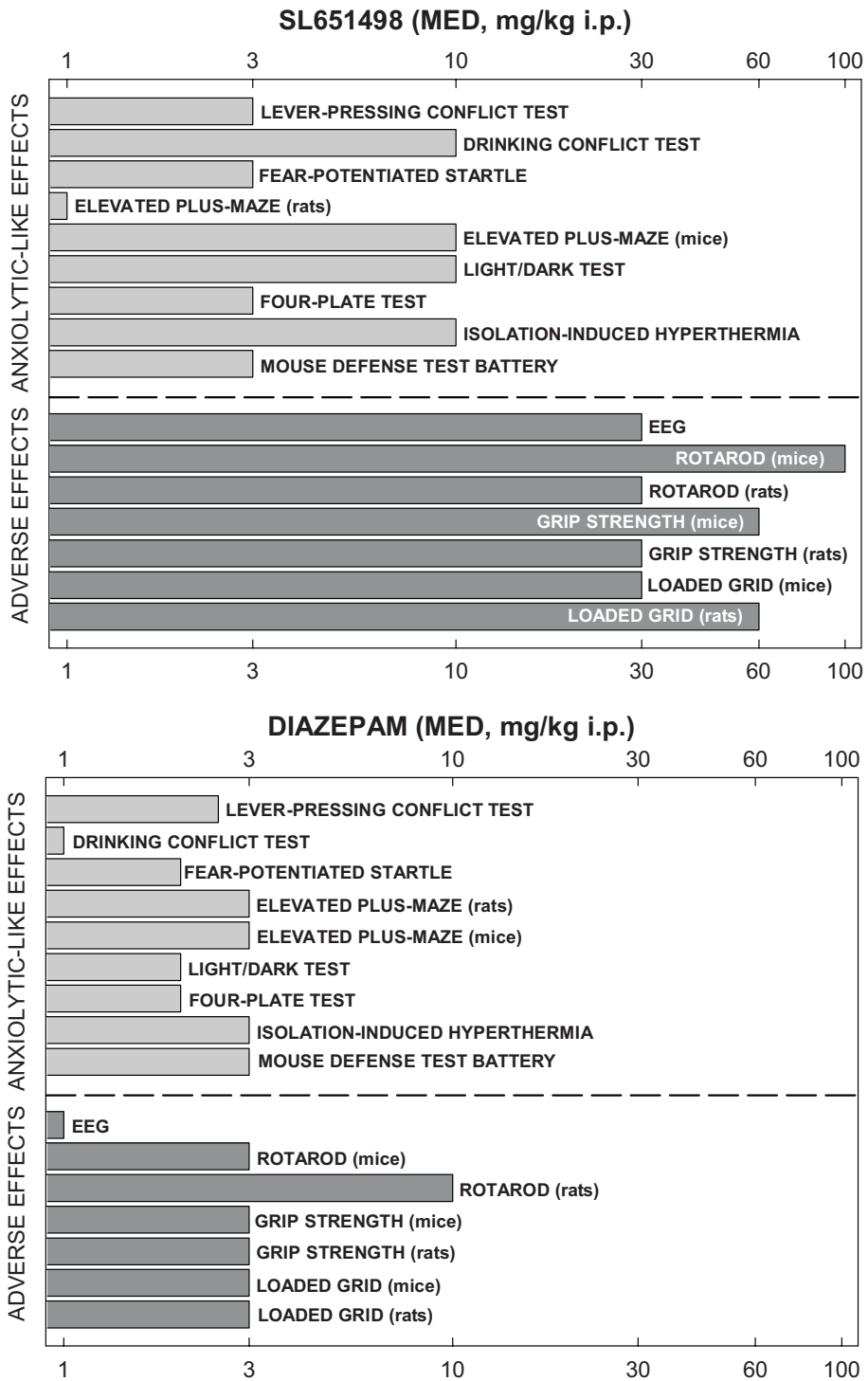
Fig. 7. Comparison of the minimal effective anxiolytic-like and central depressant doses obtained with SL651498 and diazepam in a variety of tests in mice and in rats.

both models at anxiolytic doses (i.e., 1 to 10 mg/kg, i.p. or p.o.). Results showed that the drug did not induce any impairment of spatial reference and working memory. This was in contrast to diazepam, which produced deleterious effects in the Morris water maze at 10 mg/kg, p.o., a dose which overlaps with the minimal effective anxiolytic dose (see Table 4). Two nonspatial memory tasks are commonly used to evaluate the effects of BZs on cognitive capacities: object recognition (47) and passive avoidance (24) response. Animals treated with BZs are generally unable to discriminate between familiar and novel objects, thereby showing impaired working memory, and have impaired performance in the passive avoidance test. While SL651498 did not significantly affect learning at doses up to 30 mg/kg, p.o. in the object recognition task in mice, it disrupted the acquisition of conditioned fear in the passive avoidance test at 10 mg/kg, i.p. However, the passive avoidance task is an averse-motivated learning test, and the impairment of acquisition of conditioned fear produced by SL651498 in this test may be confounded by its anxiolytic-like activity. Together, the finding that SL651498 is devoid of memory-impairing effects at anxiolytic doses suggests a low liability of the drug to produce cognitive deficits in humans.

Interaction with Alcohol

Considerable evidence indicates that some of the effects of ethanol are mediated by an action on the $GABA_A$ receptor chloride channel complex and BZs potentiate the depressant effects of ethanol [e.g., (29)]. For example, in the horizontal wire test in mice, diazepam was found to potentiate the depressant action of ethanol at a dose as low as 1 mg/kg, p.o. In the same test, SL651498 potentiated ethanol effects only at 30 mg/kg, p.o. While in the case of SL651498 this effect appeared at a dose 3 to 10 times higher than the minimal effective anxiolytic dose, diazepam produced additive effects with ethanol at doses that overlapped with those eliciting anxiolytic-like activity. Moreover, the magnitude of the potentiation of the depressant effects of ethanol was less with SL651498 than with diazepam.

Tolerance and Physical Dependence

Long-term administration of BZs is often associated with the development of tolerance. Drug tolerance has been defined as the process by which the effects of the same dose of a drug decrease with repeated administration. These effects are particularly well established for anticonvulsant and central depressant activities, but are not observed frequently in tests that assess anxiolytic-like activity (23). An experiment with SL651498 showed that the drug did not give rise to tolerance to its anticonvulsant effects against isoniazid-induced convulsions in mice following repeated administration. In contrast, marked tolerance was observed with diazepam (Fig. 8).

Numerous studies have documented physiological withdrawal syndromes following abrupt discontinuation of long-term BZ treatment (23). During withdrawal, the original anxiety symptoms often return in a more intense form. This phenomenon has also been described in laboratory research (51). In animals, BZ-induced withdrawal signs can be quantified with a variety of behavioral and physiological measures and range from convulsions to subtle behavioral changes indicative of increased anxiety. We used increased sensitivity to a convulsant drug as a measure of physiological dependence and showed that
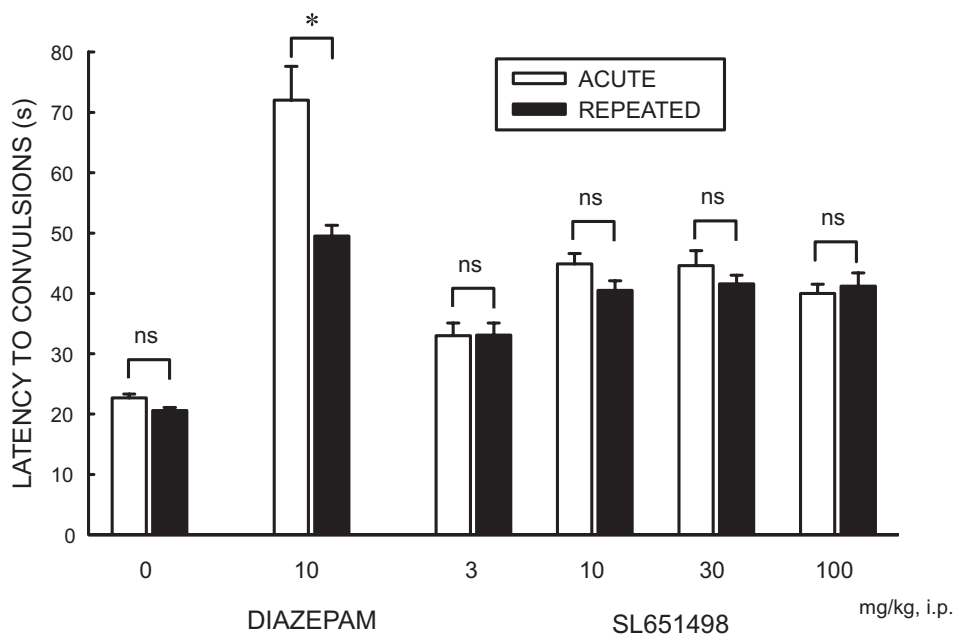
Fig. 8. Effects of repeated treatment with SL651498 or diazepam on the potency of these drugs to increase the latency to convulsions induced by isoniazid. SL651498 or diazepam was given simultaneously with isoniazid (800 mg/kg, s.c.) 42 h after the last administration of the repeated treatment with SL651498 (2×30 mg/kg, p.o.) or diazepam (2×5 mg/kg, p.o.) for 10 consecutive days. Data represent mean $\pm$ S.E.M. in mice repeatedly treated with vehicle. *$P < 0.05$; ns, non significant. Adapted from ref. 19.

repeated treatment with SL651498 for 10 days did not modify sensitivity to convulsions induced by isoniazid. By contrast, in this study, diazepam produced increased sensitivity to the convulsant challenge (19).

It is likely that the lack of occurrence of tolerance and physical dependence following repeated administration of SL651498 may be due to its weak activity and/or partial agonist activity at the GABA$_A$ receptor α_1 and/or α_5 subtypes (40). Indeed, a similar lack of tolerance to anticonvulsant action or increased sensitivity to seizures induced by convulsant challenge has been reported previously. It occured following chronic administration of ligands that have no affinity for the GABA$_A$ receptor α_5 subtype (e.g., zolpidem, zaleplon) or those that behave as partial agonists at this receptor (e.g., abecarnil, CL218,872) (44,45,53,59).

CONCLUSIONS

From the data presented in this review, SL651498 may find utility in a number of therapeutic areas, including generalized anxiety disorder and muscle spasms associated with musculoskeletal disorders. In animal experiments, SL651498 produced anxiolytic-like activity and skeletal muscle relaxant effects qualitatively and quantitatively similar to those of BZs. Unlike BZs, it induced central depressant effects at doses much higher than those

producing positive effects, suggesting that it should be devoid of sedative effects at anxiolytic/muscle relaxant doses. Moreover, in contrast to BZs, SL651498 did not produce tolerance to its anticonvulsant activity or physical dependence. SL651498 was much less active than BZs in potentiating the depressant effects of ethanol or impairing cognitive processes in rodents, which indicates a very low liability of this compound to induce classical BZ side effects. The anxioselective profile of SL651498 compared to BZs may be related to its different intrinsic efficacy and/or selectivity for certain GABA$_A$ receptor subtypes. This suggests that selectively targeting GABA$_A$ receptor subtypes can lead to drugs with increased clinical specificity (31). SL651498 represents a promising alternative to agents currently used for the treatment of anxiety and muscle spasms without the major side effects seen with classical BZs.

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