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Comparison of the pharmacological properties of classical and novel BZ-ω receptor ligands

G. Griebel, G. Perrault, S. Tan, H. Schoemaker and D.J. Sanger

CNS Research Department, Sanofi-Synthélabo, Bagneux, France

Correspondence to G. Griebel. CNS Research Department, Sanofi–Synthélabo, 31 avenue Paul Vaillant-Couturier, 92220 Bagneux, France. E-mail:ggriebel@bagneux.synthelabo.fr

The experiments in this study compared the pharmacological properties of several BZ-ω receptor ligands, including the imidazobenzodiazepine imidazenil, the β-carboline abecarnil, the pyridazinone Y-23684, the pyrido[1,2-a]benzimidazole RWJ 46771 and the 1,6-naphthyridin-2(1H)-one derivative SX-3228, with the prototypical BZs diazepam, clobazam and bretazenil. In *in vitro* experiments diazepam, bretazenil, imidazenil and Y-23684 displaced [^{3}H]flumazenil binding non-selectively in membranes from rat cerebellum and spinal cord, two brain areas enriched in the BZ-ω_1 and BZ-ω_2 receptor subtypes, respectively. In contrast, abecarnil, RWJ 46771 and SX-3228 were more potent in displacing [^{3}H]flumazenil binding to membranes from rat cerebellum than from spinal cord or hippocampus, indicating selectivity for the BZ-ω_1 receptor subtype. The *in vivo* experiments showed that all compounds increased the latency to clonic seizures produced by isoniazid. However, the maximal increase in latency induced by diazepam, clobazam, abecarnil, RWJ 46771 and SX-3228 was greater than that of bretazenil, imidazenil and Y-23684, thereby indicating that these latter compounds have low intrinsic efficacy. In the punished drinking, the punished lever pressing and the elevated plus-maze tests in rats, three models of anxiety, diazepam, clobazam and imidazenil elicited clear anxiolytic-like effects but at doses which were close to those producing hypolocomotion, ataxia and myorelaxation as measured in activity cages, the rotarod and the loaded grid tests, respectively. In contrast, bretazenil and Y-23684 induced anxiolytic-like activity at much lower doses than those which impaired motor performances. The magnitude of the positive effects of Y-23684 was similar to that of the reference BZs, suggesting that it may become a valuable alternative to currently used agents for the treatment of anxiety disorders. Abecarnil, RWJ 46771 and SX-3228 produced weaker or non-specific anxiolytic-like effects as they decreased anxiety-related behaviours at doses similar or close to those impairing motor performance. However, unlike the other compounds they induced myorelaxation at doses which were 3–10 times higher than those needed to produce decrease in exploratory activity. It is suggested that the behavioural profiles of abecarnil, RWJ 46771 and SX-3228 may be attributed to their selectivity for the BZ-ω_1 receptor subtype which may account for their sedative activity, thereby masking other effects including anxiolytic-like activity. This suggests that BZ receptor modulation of anxiety may involve BZ receptor subtypes other than BZ-ω_1.

Keywords: abecarnil, anxiety, ataxia, binding, benzodiazepines, convulsions, imidazenil, myorelaxation, omega receptors, RWJ 46771, SX-3228, Y-23684, rat, mouse

INTRODUCTION

Benzodiazepines (BZs) are the most frequently used psychotropic agents and are widely prescribed for the treatment of anxiety and sleep disorders. Even though BZs are relatively safe drugs, they may produce untoward side-effects such as sedation, muscle relaxation, memory impairment, tolerance and physical dependence (Lader, 1994). BZs produce their pharmacological effects by allosterically and positively modulating the action of GABA at $GABA_A$ receptors at two specific ionotropic sites initially described as BZ_1 and BZ_2 (Squires *et al.*, 1979; Sieghart and Schuster, 1984), also designated as ω_1 and ω_2, respectively (Langer and Arbilla, 1988). $GABA_A$ receptors have a pentameric structure formed from members of at least five different subunit families (α_{1-6}, β_{1-3}, γ_{1-3}, ρ_{1-2} and δ_1). It is now widely acknowledged that the BZ-ω_1 subtype corresponds to receptors containing the α_1-subunit, while the BZ-ω_2 subtype represents a heterogeneous population of sites possessing α_2, α_3 or α_5-subunits (for reviews, see Luddens *et al.*, 1995; Sieghart, 1995).

The search for compounds chemically unrelated to BZs, with more specific therapeutic actions without the concomitant unwanted effects, has led to the development of drugs that either bind selectively to specific BZ-ω receptor subtypes (e.g. zolpidem,

abecarnil) (Depoortere *et al.*, 1986; Langer *et al.*, 1990; Stephens *et al.*, 1990) and/or show different efficacies at BZ-ω receptors (e.g. bretazenil, imidazenil, abecarnil) (Martin *et al.*, 1988; Stephens *et al.*, 1990; Giusti *et al.*, 1993). For example, studies in animals showed that the non-selective BZ-ω receptor partial agonists bretazenil and imidazenil displayed comparable or even greater efficacy in anxiety models than BZs, but produced less motor impairment (Martin *et al.*, 1988; Cole and Rodgers, 1993; Giusti *et al.*, 1993; Jones *et al.*, 1994; Sanger, 1995; Sanger *et al.*, 1995; Griebel *et al.*, 1996a). Furthermore, the selective BZ-ω_1 receptor agonists zolpidem and abecarnil were found to produce sedative activity at much lower doses than those producing ataxia and myorelaxation (Depoortere *et al.*, 1986; Perrault *et al.*, 1990; Stephens and Voet, 1994), and after repeated treatment, zolpidem and abecarnil did not produce tolerance and physical dependence in rodents, as was observed with most BZs (Perrault *et al.*, 1992; Zivkovic *et al.*, 1992; Serra *et al.*, 1994a). More recently it was shown that the pyridazinone derivative Y-23684, a positive modulator of BZ-ω receptors, displayed a selective anxiolytic-like profile in rodents with lower liabilities for sedation and muscle relaxation compared to conventional BZs (Yasumatsu *et al.*, 1994). Whereas Y-23684 is described as BZ-ω receptor partial agonist, abecarnil acts as a full agonist at BZ-ω sites associated with $GABA_A$ receptors containing α_1- or α_3-subunits and as a partial agonist at sites associated with $GABA_A$ receptors with α_2- or α_5-subunits (Knoflach *et al.*, 1993; Pribilla *et al.*, 1993; Wafford *et al.*, 1993; Yasumatsu *et al.*, 1994). The pyrido[1,2-a]benzimidazole derivative RWJ 46771, which binds with subnanomolar affinity (IC_{50} = 0.42 nM) to the BZ site of the $GABA_A$ receptor, has been shown to display potent anticonflict activity in the punished drinking test in rats (Maryanoff *et al.*, 1995). It has been reported that the 1,6-naphthyridin-2(1H)-one derivative and selective BZ-ω_1 receptor agonist SX-3228, although less effective as the BZ triazolam in anxiety tests (Griebel *et al.*, 1998), has potent hypnotic and anticonvulsant effects with little muscle relaxant activity (Ohta, 1996).

The present experiments aimed at comparing the effects of abecarnil, imidazenil Y-23684, RWJ 46771 and SX-3228 under identical test conditions in several rat models of anxiety, including conflict procedures (punished lever-pressing and Vogel drinking tests) and an exploratory model (elevated plus-maze test). The specificity of drug response was additionally examined in separate groups of animals by measuring spontaneous locomotion in activity cages, ataxia in the rotarod test and myorelaxation in the loaded grid test. Effects were directly compared to those of diazepam, clobazam and bretazenil, which were used throughout as positive controls. The drugs' anticonvulsant actions against isoniazid-induced seizures were also examined. This latter test was used in order to assess the intrinsic activity of the compounds at $GABA_A$ receptors (Mao *et al.*, 1975). In addition, a binding profile to native BZ-ω receptor subtypes was established for each drug.

METHODS

All procedures described here are in compliance with French legislation on research with animals.

Subjects

Male Wistar rats (Charles River France, Saint-Aubin-les-Elbeuf) were used in the punished lever-pressing procedure. They weighed 180–200 g at the beginning of training and 400–500 g at the time of drug testing. Male Sprague–Dawley rats (Iffa Credo, L'Arbresle and Charles River France) weighing 180–300 g at time of testing were used in the Vogel drinking procedure, the elevated plus-maze test, the actimeter, the rotarod and the loaded grid tests, and for radioligand binding studies. Rats used in the Vogel drinking procedure, the elevated plus-maze test, the actimeter, the rotarod and the loaded grid tests, and those used in radioligand binding studies, were housed in groups of eight, whereas those used in the punished lever-pressing procedure were housed singly. Male CD1 mice (Charles River France, Saint-Aubin-les-Elbeuf) weighing 18–25 g and housed in groups of 20 were used in the isoniazid-induced convulsions test. All animals were maintained under standard laboratory conditions (22–23°C) and kept on a 12 h light–12 h dark cycle, with light onset at 07.00 h. Rats used in the punished lever-pressing procedure were restricted to the food obtained during sessions and a daily ration of 15–20 g of standard laboratory chow given at the end of each weekday and over the weekend. With the exception of the punished lever-pressing test, animals were only used once. Testing was performed between 09.00 and 15.00 h.

Behavioural procedures

Punished lever pressing

The procedure was a modification of that described previously (Sanger *et al.*, 1985). Animals were tested in standard rat operant test chambers (MED Associates Inc., St Albans, VT, USA) placed in sound-attenuated boxes with ventilation fans. Each

chamber was fitted with a stainless steel grid floor. Electric shocks could be delivered to each grid by a shock generator and scrambler (MED Associates Inc.). A total of 11 rats were trained initially to press a lever for food reward (45 mg precision food pellets; P.J. Noyes Inc., Lancaster, NH, USA). As training progressed, schedule parameters were gradually changed to a variable-interval (VI 30 s) schedule of food reinforcement during daily 15-min sessions. After several sessions of VI 30 s responding, five 60-s periods of a visual stimulus were presented during a 25-min session. Each visual stimulus consisted of three stimulus lights situated above the food-pellet dispenser and to the right of the response lever, which flashed at a rate of 1 s on, 1 s off. In this component, a footshock punishment schedule consisting of two independent VI schedules (VI 30 s for food, VI 10 s for shock) was in operation. Footshock was initially set at 0.1 mA. The first stimulus presentation started 5 min after the beginning of the session, and each following stimulus commenced 150 s after the end of the preceding stimulus. The magnitude of footshock was individually titrated for each rat (shock levels ranged from 0.3 to 0.65 mA) to obtain stable baselines of responding (i.e. an average lever-pressing rate of 8 ± 2 presses in each 60-s punished responding period). To obtain stable levels of responding, an average of approximately 30 sessions after initiation of the punishment contingency was necessary. Once stable baselines of responding were obtained, drug studies were initiated.

Drug injections were given once or twice each week with at least two non-drug days intervening between two drug administrations. Vehicle was injected on all non-drug days. Drugs and doses were given in a mixed order. The effects of drugs were assessed on punished and unpunished responses rates. The former corresponds to those recorded during the presentation of the visual stimulus, whereas the latter were taken from the 60-s periods immediately preceding and immediately following each stimulus presentation. The mean values of punished and unpunished rates recorded during the non-drug session preceding the drug-injection sessions were used as the control values. Thus, drug effects were analysed statistically by comparing performances after drug administration with the mean values taken from appropriate control sessions using a Friedman's ANOVA.

Punished drinking

The procedure was a modification of the technique described by Vogel *et al.* (1971). At the beginning of the experiment rats, deprived of water for 48 h prior to testing, were placed in cages (27 × 22 × 21 cm) with a stainless steel grid floor. Each cage contained a drinking tube connected to an external 50-ml burette filled with tap water. Trials were started only after the animal's tongue became in contact with the drinking tube for the first time. An electric shock (0.06 mA) was delivered to the tongue after every 20 licks. The number of shocks was recorded automatically during a 3-min period. Data were analysed with one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

Elevated plus-maze

The test apparatus is based on that described by Pellow *et al.* (1985). All parts of the apparatus were made of dark polyvinylplastic with a black rubber floor. The maze was elevated to a height of 50 cm with two open (50 × 10 cm) and two enclosed arms (50 × 10 × 50 cm), arranged so that the arms of the same type were opposite each other, connected by an open central area (10 × 10 cm). To prevent rats falling off, a rim of Plexiglas (1 cm high) surrounded the perimeter of the open arms. The illumination in the experimental room consisted of one red neon tube fixed on the ceiling, so that experiments were performed under dim light conditions. The light intensity on the central platform was 10 lux. At the beginning of the experiment rats were placed in the centre of the maze, facing one of the enclosed arms, and observed for 4 min. The apparatus was equipped with infrared beams and sensors capable of measuring time spent in open arms, number of open-arm entries and number of closed-arm entries (defined as entry of all four limbs into an arm of the maze). In addition, rats were observed via video-link by an observer located in an adjacent room. This permitted the recording of the more ethologically orientated measures: (a) *attempt* – attempt at entry into open arms followed by avoidance responses (this risk-assessment measure includes stretched-attend posture: the rat stretches forward and retracts to original position); and (b) *head-dipping* – protruding the head over the edge of an open arm and down towards the floor (this response can occur while the animal's body is in a closed arm, central square or on an open arm). The results were expressed as mean ratio of time spent in open arms to total time spent in both open and closed arms, mean ratio of entries into open arms to total entries into both open and closed arms, mean total number of both closed and open arm entries, mean total number of closed arm entries, mean total

number of attempts and mean total number of head-dips. Data were analysed with one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

The actimeter

Testing was conducted in square, clear Plexiglas boxes (40 × 40 × 15 cm) equipped with infrared beams and sensors, and placed in sound-attenuated cupboards. Horizontal locomotor activity was quantified as total number of beams crossed during a 20-min period. Immediately after injection, a subject was placed in the centre of the apparatus. Data were analysed by a one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

The rotarod

Rats were trained to stay on a rotarod turning at a speed of 5 turns/min (diameter 6 cm) for at least 1 min. It was not necessary to eliminate any animals. Approximately 3 h later a second pre-test was given during which the apparatus first turned at the same constant speed for 1 min and then turned at an accelerating speed (5–42 turns/min in 10 min). Twenty-four hours later, each rat was injected with the drugs and, 30 min later, was replaced on the rotarod. The length of time each animal stayed on the apparatus at accelerating speed was recorded. Data were analysed by a one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

The loaded grid

Changes in grip strength were measured before and after drug administration by noting the maximum tolerated weight suspended from a grid gripped by the forepaws of a rat lifted by the tail. Data were analysed by a one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

Isoniazid-induced convulsions

Isoniazid (800 mg/kg, s.c.) was administered simultaneously with the test drugs. The anticonvulsant effect was assessed by measuring the latency to the appearance of the first convulsion. Isoniazid is an inhibitor of glutamic acid decarboxylase, the enzyme responsible for GABA synthesis. Data were analysed using a one-way ANOVA followed by a Dunnett's *t*-test.

In vitro binding to different BZ-ω receptor subtypes

Inhibition of [^{3}H]flumazenil binding to native BZ-ω receptor subtypes *in vitro* was studied as described by Schoemaker *et al.* (1997). Briefly, the cerebellum, spinal cord or hippocampus of rats were homogenized in 50 mM Tris–HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl. The binding of [^{3}H]flumazenil (1 nM; specific activity 70–87 Ci/mmol) to the ω_1 receptor was studied in membranes from the rat cerebellum, a region enriched in this receptor subtype (Braestrup and Nielsen, 1980), using a 45-min incubation at 0–4°C and 1 mM diazepam to define non-specific binding. [^{3}H]Flumazenil binding to the ω_2 receptor was studied using membranes from the rat spinal cord, where a majority of the expressed ω receptors appear to be of the ω_2 subtype (Ruano *et al.*, 1992), under otherwise identical conditions. The native ω_5 receptor was studied using [^{3}H]flumazenil binding to membranes from the rat hippocampus in the presence of 5 μM zolpidem in order to mask the ω_1 and ω_2 receptor subtypes (Tan and Schoemaker, 1994), otherwise under identical conditions except for the use of 1 mM flunitrazepam to define non-specific binding. Following incubation, membranes were recovered by vacuum filtration over Whatman GF/B filters, washed and the amount of radioactivity retained on the filter was quantified by liquid scintillation spectrometry. Data are presented as the compound concentration required to inhibit 50% of specific radioligand binding (IC_{50}).

Drugs

All drugs were prepared as solutions or suspensions in physiological saline containing one or two drops of Tween 80. They were injected in a volume of 2 ml/kg (rats) or 20 mg/kg (mice). The drugs used were diazepam, chlordiazepoxide HCl, clobazam, flunitrazepam, zolpidem tartrate, bretazenil, imidazenil, RWJ 46771, SX-3228 (synthesized by the Department of Chemistry, Synthélabo Recherche), abecarnil (courtesy of Schering), Y-23684 (courtesy of Yoshitomi Pharmaceutical Industries, Osaka, Japan) and isoniazid (Sigma Chemicals, St. Louis, MO, USA). [^{3}H]Flumazenil was purchased from NEN Life Science Products, (Paris, France). Y-23684 was administered i.p. 60 min before testing was performed and all other drugs were given 30 min before *in vivo* experiments. Doses are expressed as the bases.

RESULTS

In vitro binding to different BZ-ω receptor subtypes

Table 1 shows a comparison of the potency of diazepam, clobazam, bretazenil, abecarnil, imidazenil, Y-23684, RWJ 46771 and SX-3228 to displace the binding of [^{3}H]flumazenil from native BZ-ω_1, BZ-ω_2

TABLE 1. Effects of several BZ receptor ligands on the binding of [^{3}H]flumazenil to the native BZ-ω_1 receptor in the cerebellum, the native BZ-ω_2 receptor in the spinal cord and the native ω_5 receptor in the hippocampus[a]

	IC_{50} (nM)		
	BZ-ω_1	BZ-ω_2	ω_5
Diazepam	19 ± 2	12 ± 2	111 ± 15
Clobazam	403 ± 83	347 ± 31	> 1000
Bretazenil	0.49 ± 0.06	0.68 ± 0.05	5.3 ± 1.6
Imidazenil	0.49 ± 0.04	0.75 ± 0.09	6.9 ± 1.4
Y-23684	190 ± 14	260 ± 20	876 ± 15
Abecarnil	1.38 ± 0.42	3.95 ± 1.6	13.1 ± 6
RWJ 46771	0.36 ± 0.04	1.03 ± 0.12	12.4 ± 2.7
SX-3228	8.9	58	295

[a]The ω_5 receptor in the hippocampus was studied in the presence of 5 μM zolpidem, in order to mask the BZ-ω_1 and BZ-ω_2 receptors. Data are presented as the mean ± SEM of at least three experiments, except SX-3228 which was tested in one experiment only.

and ω_5 receptors in cerebellar, spinal cord and hippocampal membranes, respectively. Unlike diazepam, clobazam, bretazenil, imidazenil and Y-23684, which bind to BZ-ω_1 and BZ-ω_2 receptors with similar half maximally effective concentrations, abecarnil, RWJ 46771 and SX-3228 show selectivity for the native BZ-ω_1 receptor. The affinities of RWJ 46771 for the native BZ-ω_1 and BZ-ω_2 receptors (IC_{50} = 0.36 and 1.03 nM) were similar to those of bretazenil and imidazenil, and higher than that of diazepam, clobazam, abecarnil, SX-3228 and Y-23684. Bretazenil and imidazenil shared similar high affinities (IC_{50} = 1.39 and 1.53 nM, respectively) for the native ω_5 receptor. These values were slightly higher than that of abecarnil and RWJ 46771, and considerably higher than that of the other compounds. All drugs showed selectivity for BZ-ω_1 relative to native ω_5 receptor in the hippocampus.

Punished lever pressing

Figure 1 shows that the rates of responding decreased by the punishment contingency were significantly increased by diazepam (χ^2 = 20.75, $P < 0.001$), clobazam (χ^2 = 15.47, $P < 0.01$), imidazenil (χ^2 = 13.05, $P < 0.001$) and Y-23684 (χ^2 = 21.69, $P < 0.001$), but not by RWJ 46771. These effects occurred at doses which did not modify unpunished responding, except for diazepam which significantly increased this measure at 2.5 mg/kg (χ^2 = 9.3, $P < 0.05$). Unpunished responding was significantly decreased by RWJ 46771 at 0.3 mg/kg (χ^2 = 18.6, $P < 0.001$).

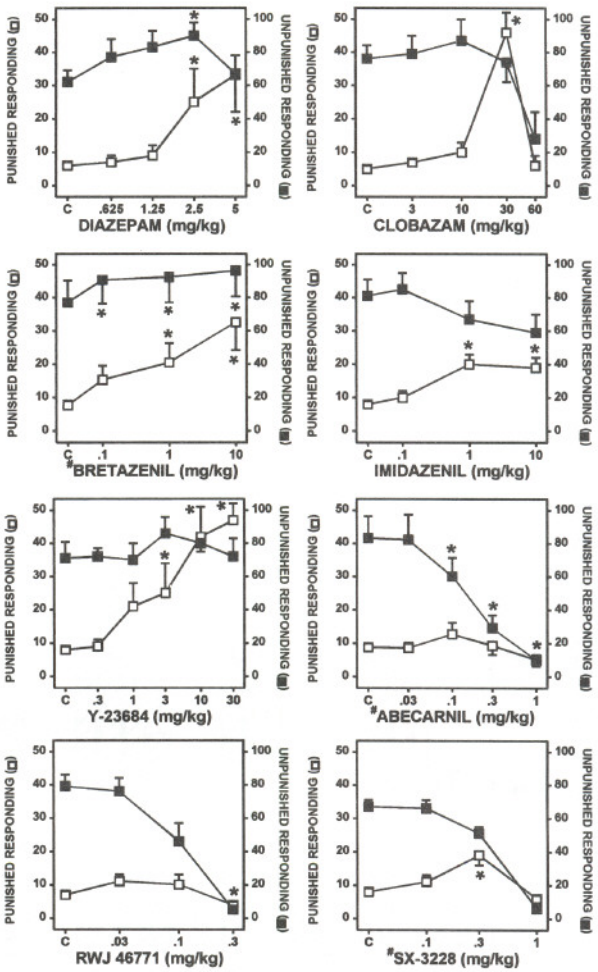

FIGURE 1. Effects of several classical and novel BZ-ω receptor ligands on rates of punished and unpunished lever pressing in rats. Drugs were administered intraperitoneally 60 (Y-23684) or 30 (all other drugs) min before testing. Data represent mean ± SEM. n = 8, *$P < 0.05$ (Friedman test). #Data obtained with bretazenil, abecarnil and SX-3228 were published in previous papers (Sanger, 1995; Griebel *et al.*, 1998).

Punished drinking

Figure 2 shows that all compounds significantly modified the number of shocks received [diazepam: $F(4,45) = 3.65$, $P < 0.05$; clobazam: $F(4,65) = 3.77$, $P < 0.01$; bretazenil: $F(4,45) = 5.61$, $P < 0.001$; abecarnil: $F(4,35) = 3.32$, $P < 0.05$; imidazenil: $F(4,44) = 2.83$, $P < 0.05$; Y-23684: $F(4,45) = 3.14$, $P < 0.05$; RWJ 46771: $F(4,45) = 2.3$, $P < 0.05$]. Post-hoc analysis indicated that while clobazam (10 and 30 mg/kg), bretazenil (1 and 10 mg/kg), imidazenil (0.3–3 mg/kg), Y-23684 (3 and 30 mg/kg) and RWJ 46771 (1 and 3 mg/kg) significantly increased punished responding at several doses, diazepam (10 mg/kg) and abecarnil (3 mg/kg) produced similar effects at the highest dose only.

Elevated plus-maze

Table 2 shows that with the exception of RWJ 46771 the drugs significantly modified both the percentage of time spent on the open arms [diazepam: $F(4,30) = 3.23$, $P < 0.05$; clobazam: $F(4,56) = 8.23$, $P < 0.001$; bretazenil: $F(4,30) = 9.66$, $P < 0.001$; abecarnil: $F(4,30) = 5.32$, $P < 0.01$; imidazenil: $F(5,64) = 13.44$, $P < 0.001$; Y-23684: $F(4,30) = 10.54$, $P < 0.001$] and the percentage of entries made into the open arms [diazepam: $F(4,30) = 4.39$, $P < 0.01$; clobazam: $F(4,56) = 9.22$, $P < 0.001$; bretazenil: $F(4,30) = 11.94$, $P < 0.001$; abecarnil: $F(4,30) = 6.7$, $P < 0.001$; imidazenil: $F(5,64) = 9.86$, $P < 0.001$; Y-23684: $F(4,30) = 8.64$, $P < 0.001$]. Post-hoc analysis indicated that clobazam (3–30 mg/kg), bretazenil (0.01–1 mg/kg), abecarnil (0.3–3 mg/kg) and imidazenil (0.1–3 mg/kg) significantly increased activity in open arms over a wide dose range. Y-23684 produced a similar effect at 1 and 10 mg/kg, whereas diazepam significantly increased open arm time and entries at the highest dose only (3 mg/kg). With respect to the ethologically derived measures, all compounds modified the number of attempts at entry into open arms followed by avoidance responses [diazepam: $F(4,30) = 4.73$, $P < 0.01$; clobazam: $F(4,56) = 16.59$, $P < 0.001$; bretazenil: $F(4,30) = 9.7$, $P < 0.001$; abecarnil: $F(4,30) = 4.06$, $P < 0.01$; imidazenil: $F(5,64) = 16.69$, $P < 0.001$; Y-23684: $F(4,30) = 8.49$, $P < 0.001$; RWJ 46771: $F(5,64) = 7.77$, $P < 0.001$]. Post-hoc analysis indicated that clobazam (1–30 mg/kg), bretazenil (0.01–1 mg/kg), abecarnil (0.3–3 mg/kg), imidazenil (0.1–3 mg/kg), Y-23684 (0.01–10 mg/kg) and RWJ 46771 (0.1–1 mg/kg) significantly reduced attempts at several dose levels, whereas diazepam decreased this behaviour at the highest dose only (3 mg/kg). In addition, diazepam [$F(4,30) = 3.7$, $P < 0.05$], clobazam [$F(4,56) = 7.09$, $P < 0.001$], bretazenil [$F(4,30) = 7.47$, $P < 0.001$], abecarnil [$F(4,30) = 2.5$,

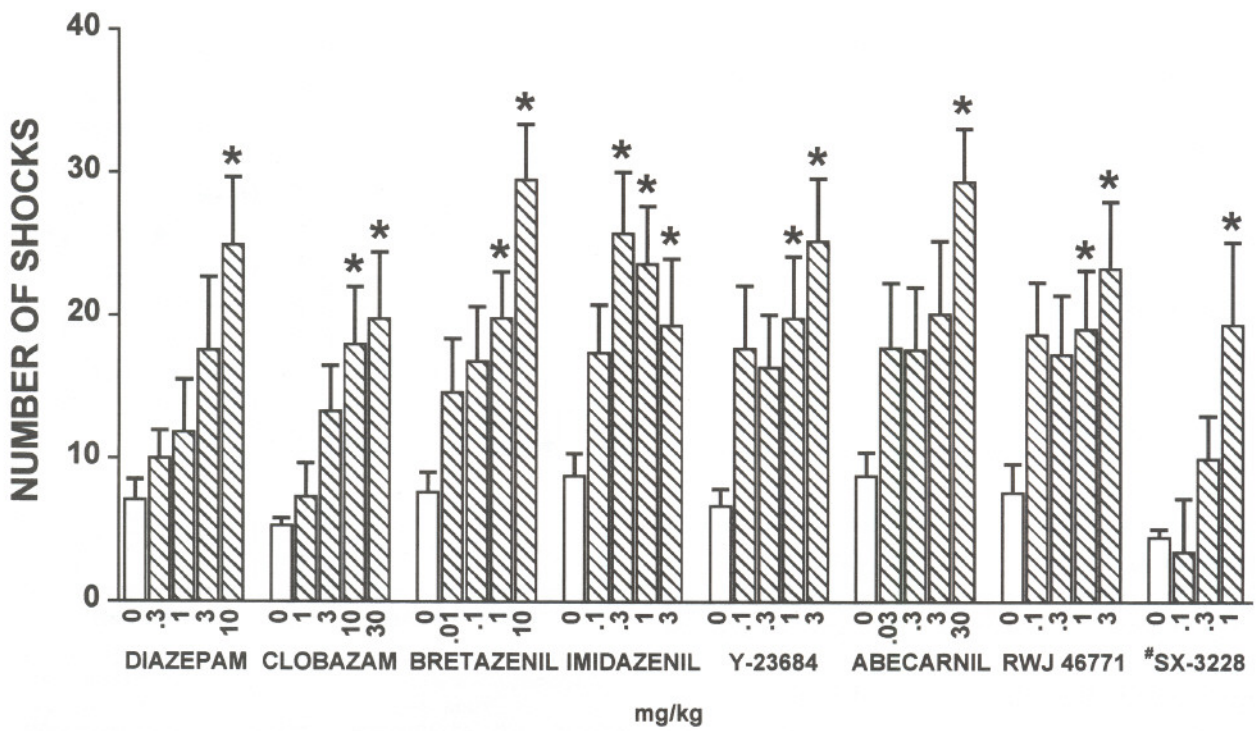

FIGURE 2. Effects of several classical and novel BZ-ω receptor ligands in the punished drinking conflict test in rats. Drugs were administered intraperitoneally 60 (Y-23684) or 30 (all other drugs) min before testing. Data represent mean ± SEM. $n = 10$, $^*P < 0.05$ (Dunnett's t-test). $^{\#}$Data obtained with SX-3228 were published in a previous paper (Griebel *et al.*, 1998).

TABLE 2. Effects of several benzodiazepine receptor ligands on the behavior of rats on the elevated plus-maze[a]

	Dose (mg/kg)	% time in open arms	% entries in open arms	Attempts	Head-dips	Closed arm entries
Diazepam	0	15.2 ± 5.1	17.3 ± 5.9	9.0 ± 1.2	4.4 ± 0.9	8.3 ± 0.8
	0.1	20.1 ± 7.1	17.0 ± 6.6	7.1 ± 0.5	5.4 ± 1.2	9.7 ± 0.8
	0.3	17.9 ± 7.8	16.4 ± 6.7	10.0 ± 0.6	5.4 ± 1.7	13.7 ± 1.3[b]
	1	33.2 ± 7.6	30.9 ± 6.0	6.9 ± 1.4	8.1 ± 1.2	11.0 ± 0.9
	3	47.8 ± 9.5[b]	50.6 ± 9.6[b]	3.6 ± 1.6[b]	13.3 ± 3.3[b]	6.0 ± 0.7
Clobazam	0	9.3 ± 3.6	10.7 ± 3.8	11.1 ± 0.7	3.4 ± 0.8	9.5 ± 0.5
	1	21.5 ± 8.6	21.8 ± 8.0	6.3 ± 1.0[b]	4.0 ± 1.0	7.6 ± 0.5
	3	28.3 ± 5.6[b]	28.7 ± 4.3[b]	5.6 ± 0.9[b]	7.9 ± 1.2[b]	8.9 ± 0.6
	10	41.3 ± 7.4[b]	37.6 ± 5.4[b]	5.2 ± 0.9[b]	11.2 ± 1.6[b]	7.5 ± 0.6
	30	53.7 ± 6.5[b]	52.1 ± 6.3[b]	1.9 ± 0.8[b]	11.4 ± 1.9[b]	7.1 ± 1.2
Bretazenil	0	11.5 ± 6.3	12.4 ± 6.0	11.7 ± 0.8	2.9 ± 0.9	9.6 ± 0.2
	0.01	10.0 ± 3.9	13.1 ± 5.0	9.0 ± 0.8[b]	3.4 ± 1.0	9.1 ± 0.5
	0.03	36.8 ± 5.9[b]	38.3 ± 4.3[b]	5.4 ± 1.0[b]	8.1 ± 1.1[b]	8.0 ± 0.6
	0.3	36.1 ± 3.2[b]	37.1 ± 2.6[b]	5.9 ± 0.7[b]	10.4 ± 1.3[b]	11.3 ± 1.1
	1	46.2 ± 6.2[b]	45.4 ± 3.7[b]	6.3 ± 0.9[b]	9.9 ± 1.8[b]	10.8 ± 1.1
Imidazenil	0	9.7 ± 2.5	14.1 ± 3.1	12.4 ± 0.7	4.8 ± 0.7	10.1 ± 0.6
	0.03	19.8 ± 8.1	21.4 ± 8.2	10.4 ± 1.0	6.4 ± 2.1	8.9 ± 0.6
	0.1	28.3 ± 4.4[b]	31.0 ± 3.9[b]	8.6 ± 1.0[b]	10.5 ± 1.3[b]	9.4 ± 0.6
	0.3	27.9 ± 4.0[b]	32.8 ± 4.2[b]	6.2 ± 1.0[b]	9.6 ± 1.3[b]	9.3 ± 0.8
	1	49.9 ± 3.8[b]	44.7 ± 3.4[b]	3.7 ± 0.4[b]	14.7 ± 1.3[b]	7.8 ± 0.6[b]
	3	46.8 ± 3.7[b]	51.7 ± 4.0[b]	3.1 ± 0.9[b]	14.7 ± 2.6[b]	6.7 ± 0.7[b]
Y-23684	0	11.5 ± 3.7	14.5 ± 3.9	12.0 ± 0.5	4.1 ± 0.6	9.0 ± 0.5
	0.01	6.8 ± 2.4	11.3 ± 3.5	8.6 ± 0.5[b]	4.0 ± 0.7	9.4 ± 0.6
	0.1	13.8 ± 4.2	16.0 ± 3.9	8.3 ± 1.3[b]	4.7 ± 1.3	9.7 ± 0.5
	1	28.6 ± 2.8[b]	34.1 ± 4.5[b]	7.6 ± 0.8[b]	9.6 ± 0.7[b]	10.9 ± 1.1
	10	34.5 ± 4.8[b]	34.3 ± 3.2[b]	4.4 ± 1.2[b]	13.4 ± 2.1[b]	10.7 ± 0.7
Abecarnil	0	8.9 ± 2.9	10.4 ± 2.4	9.1 ± 0.7	3.1 ± 1.2	8.3 ± 0.8
	0.1	22.1 ± 6.6	21.2 ± 6.1	8.0 ± 1.1	5.6 ± 1.5	9.7 ± 0.8
	0.3	34.2 ± 8.8[b]	33.2 ± 7.2[b]	3.7 ± 0.7[b]	7.7 ± 2.2[b]	13.7 ± 1.3[b]
	1	31.4 ± 3.5[b]	44.8 ± 7.4[b]	5.3 ± 1.3[b]	8.7 ± 1.3[b]	11.0 ± 0.9
	3	52.9 ± 10.0[b]	57.0 ± 10.2[b]	5.4 ± 1.5[b]	8.1 ± 1.5[b]	6.0 ± 0.7
RWJ 46771	0	7.6 ± 2.0	11.1 ± 2.3	10.6 ± 0.8	3.3 ± 0.5	8.9 ± 0.6
	0.01	5.9 ± 4.6	6.7 ± 3.6	9.0 ± 0.8	2.6 ± 1.6	8.3 ± 1.1
	0.03	14.6 ± 4.0	16.6 ± 4.0	7.9 ± 0.6	5.7 ± 0.8	8.1 ± 0.7
	0.1	16.5 ± 4.2	20.0 ± 4.7	7.0 ± 1.2[b]	7.1 ± 1.4[b]	7.9 ± 0.7
	0.3	18.8 ± 3.4	21.1 ± 3.5	5.4 ± 0.9[b]	6.1 ± 1.2	7.9 ± 0.9
	1	25.3 ± 11.7	23.5 ± 10.2	1.9 ± 1.7[b]	1.9 ± 0.7	3.7 ± 1.5[b]
SX-3228[c]	0	17.0 ± 5.7	15.6 ± 4.8	8.5 ± 1.0	3.2 ± 0.6	9.5 ± 0.8
	0.1	36.0 ± 4.0	33.1 ± 2.9	6.7 ± 0.7	8.1 ± 1.2[b]	8.3 ± 0.5
	0.3	41.3 ± 5.1[b]	46.2 ± 5.1[b]	3.8 ± 0.5[b]	8.2 ± 1.1[b]	5.7 ± 0.6[b]
	1	41.2 ± 7.5[b]	48.6 ± 6.2[b]	0.8 ± 0.3[b]	5.3 ± 1.1	4.3 ± 1.0[b]

[a]Y-23684 was administered 60 min before the beginning of the test. The other drugs were injected 30 min before the test. Data represent mean ± SEM. $n = 7–14$ [b]$P < 0.05$ (Dunnett's t-test, vs vehicle control). [c]Data obtained with SX-3228 were published in a previous paper (Griebel *et al.*, 1998).

$P < 0.05$], imidazenil [$F(5,64) = 8.33$, $P < 0.001$], Y-23684 [$F(4,30) = 11.97$, $P < 0.001$] and RWJ 46771 [$F(5,64) = 3.27$, $P < 0.05$] modified directed exploration (head-dips). Post-hoc analysis revealed that this response was significantly increased by clobazam (3–30 mg/kg), bretazenil (0.03–1 mg/kg), abecarnil (0.3–3 mg/kg), imidazenil (0.1–3 mg/kg) and Y-23684 (1–10 mg/kg) at several doses, whereas it was increased by diazepam at the highest dose only (3 mg/kg) and by RWJ 46771 at an intermediate dose (0.1 mg/kg). Finally, diazepam [$F(4,30) = 10$, $P < 0.001$], imidazenil [$F(5,64) = 2.7$, $P < 0.05$], abecarnil [$F(4,30) = 3.6$, $P < 0.05$] and RWJ 46771 [$F(5,64) = 3.4$, $P < 0.01$] significantly modified the total number of arm entries. While diazepam (0.3 mg/kg) and abecarnil (0.3 mg/kg) increased this behaviour, imidazenil (3 mg/kg) and RWJ 46771 reduced it at the highest dose (1 mg/kg).

The actimeter

Figure 3 shows that locomotor activity was significantly modified by all compounds [diazepam: $F(5,42)$

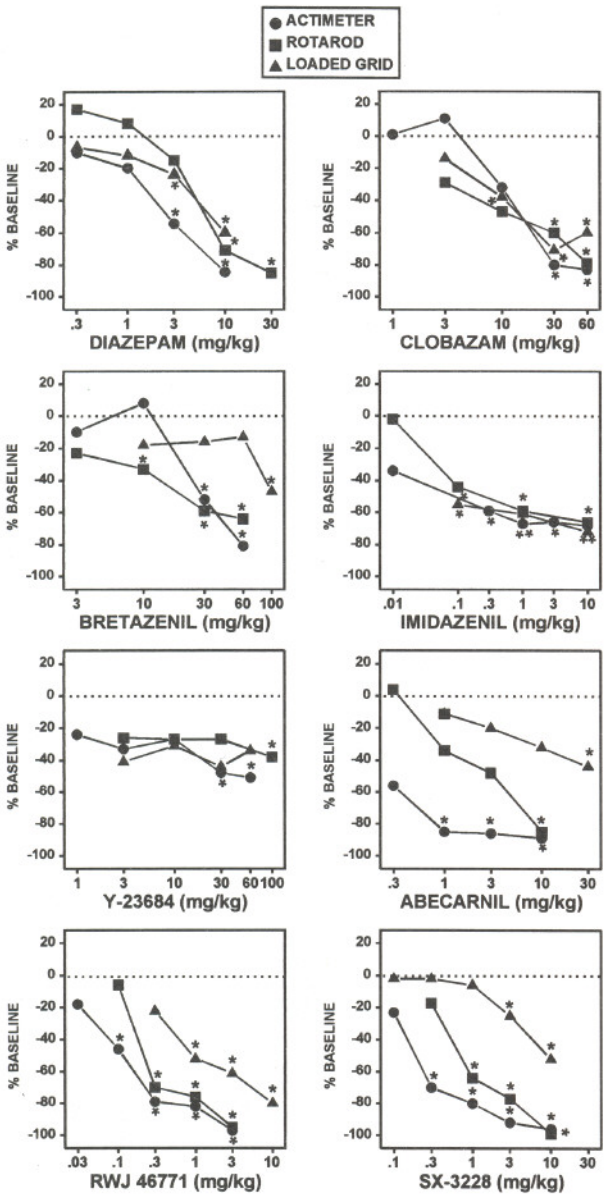

FIGURE 3. Effects of several classical and novel BZ-ω receptor ligands on tests measuring spontaneous locomotor activity (actimeter), ataxia (rotarod) and myorelaxation (loaded grid) in rats. Drugs were administered intraperitoneally 60 (Y-23684) or 30 (all other drugs) min before testing. Data are expressed as percentage of baseline levels. $n = 10$, $^*P < 0.05$ (Dunnett's t-test).

$= 25.14$, $P < 0.001$; clobazam: $F(5,42) = 22.72$, $P < 0.001$; bretazenil: $F(5,41) = 11.54$, $P < 0.001$; abecarnil: $F(5,42) = 27.29$, $P < 0.001$; imidazenil: $F(5,42) = 7.99$, $P < 0.001$; Y-23684: $F(5,42) = 4.12$, $P < 0.001$; RWJ 46771: $F(5,42) = 20.05$, $P < 0.001$; SX-3228: $F(5,42) = 41.96$, $P < 0.001$]. Dunnett comparisons indicated that diazepam (3 and 10 mg/kg), clobazam (30 and 60 mg/kg), bretazenil (30 and 60 mg/kg), abecarnil (1–30 mg/kg), imidazenil (0.3–10 mg/kg), Y-23684 (30 and 60 mg/kg), RWJ 46771 (0.1–3 mg/kg) and SX-3228 (0.3–10 mg/kg) significantly decreased the number of beams crossed.

The rotarod

Figure 3 shows that all drugs significantly impaired rotarod performance [diazepam: $F(5,36) = 9.41$, $P < 0.001$; clobazam: $F(4,35) = 4.98$, $P < 0.01$; bretazenil: $F(4,40) = 12.39$, $P < 0.001$; abecarnil: $F(4,72) = 15.61$, $P < 0.001$; imidazenil: $F(4,40) = 12.86$, $P < 0.001$; Y-23684: $F(4,30) = 14.16$, $P < 0.001$; RWJ 46771: $F(4,30) = 2.7$, $P < 0.05$; SX-3228: $F(5,36) = 26.13$, $P < 0.001$]. Subsequent analysis showed that diazepam (10 and 30 mg/kg), clobazam (30 and 60 mg/kg), bretazenil (10–60 mg/kg), abecarnil (1–10 mg/kg), imidazenil (0.1–10 mg/kg), Y-23684 (100 mg/kg), RWJ 46771 (0.3–3 mg/kg) and SX-3228 (1–30 mg/kg) significantly reduced the time on the rotarod.

The loaded grid

Figure 3 shows that all drugs except Y-23684 produced significant performance deficits [diazepam: $F(4,25) = 19.08$, $P < 0.001$; clobazam: $F(4,25) = 10.72$, $P < 0.001$; bretazenil: $F(4,25) = 8.54$, $P < 0.001$; abecarnil: $F(5,58) = 3.53$, $P < 0.01$; imidazenil: $F(4,25) = 14.61$, $P < 0.001$; RWJ 46771: $F(4,25) = 13.62$, $P < 0.001$; SX-3228: $F(5,29) = 32.08$, $P < 0.001$]. Maximum tolerated weight was decreased by diazepam and SX-3228 from 3 mg/kg, by clobazam and abecarnil from 10 mg/kg, by bretazenil at 100 mg/kg, by imidazenil from 0.1 mg/kg and by RWJ 46771 from 1 mg/kg.

Isoniazid-induced convulsions

Figure 4 shows that all compounds significantly increased the latencies to isoniazid-induced convulsions [diazepam: $F(5,53) = 83.02$, $P < 0.001$; clobazam: $F(4,45) = 136.08$, $P < 0.001$; bretazenil: $F(8,81) = 19.85$, $P < 0.001$; abecarnil: $F(6,63) = 51.95$, $P < 0.001$; imidazenil: $F(5,54) = 46.01$, $P < 0.001$; Y-23684: $F(4,45) = 40.79$, $P < 0.001$; RWJ 46771: $F(6,63) = 31.76$, $P < 0.001$; SX-3228: $F(4,45) = 24.87$, $P < 0.001$]. Post-hoc analysis revealed that the latencies to convulsions were significantly increased by diazepam, clobazam, Y-23684 and SX-3228 from 1 mg/kg, by abecarnil from 0.3 mg/kg, by bretazenil and imidazenil from 0.1 mg/kg and by RWJ 46771 from 0.03 mg/kg. The maximum increases in the latencies were greater with diazepam, clobazam, abecarnil, RWJ 46771 and SX-3228 (from 60 to

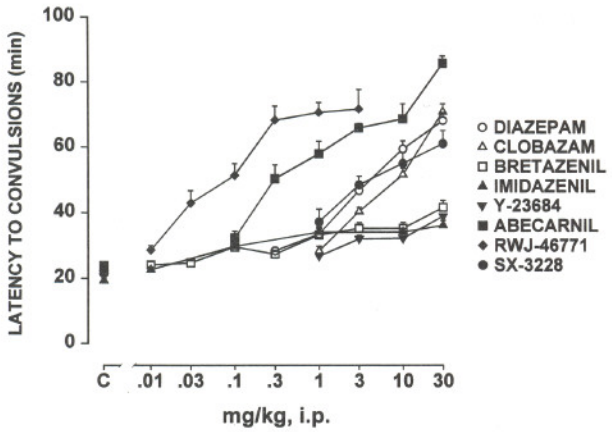

FIGURE 4. The anticonvulsant effects of several classical and novel BZ-ω receptor ligands against clonic seizures produced in mice by 800 mg/kg, s.c., of isoniazid. Data represent mean ± SEM. $n = 10$, $^*P < 0.05$ (Dunnett's t-test).

85 min) than with bretazenil, imidazenil and Y-23684 (about 40 min).

DISCUSSION

The present series of experiments aimed at comparing the biochemical and pharmacological profiles of several novel BZ-ω receptor agonists with those of the well-established anxiolytics, diazepam and clobazam, and the prototypical BZ-ω receptor partial agonist bretazenil.

The *in vitro* binding experiments to different BZ-ω receptor subtypes showed that diazepam, bretazenil, imidazenil and Y-23684 displaced [^{3}H]flumazenil binding to $GABA_A$ receptor in membranes from rat cerebellum and spinal cord, two brain areas enriched in the BZ-ω_1 and BZ-ω_2 receptor subtypes, respectively, with nearly equivalent half maximally effective concentrations. These results are in agreement with those of previous studies which showed that diazepam, bretazenil and imidazenil displayed nearly similar affinity for the BZ-ω_1 and the BZ-ω_2 receptor subtypes (Giusti *et al.*, 1993; Pribilla *et al.*, 1993), and indicate that Y-23684 recognizes both $GABA_A$ receptor subtypes with equal affinity. However, it should be noted that unlike the other non-selective compounds, Y-23684 displayed only moderate affinities for all $GABA_A$ receptor subtypes studied. The finding that Y-23684 displays no selectivity contrasts with a previous study using radioligand displacement of [^{3}H]flunitrazepam binding to synaptosomal membranes from rat cerebellum and spinal cord indicating more potent displacement by Y-23684 for the BZ-ω_1 than for the BZ-ω_2 receptor subtype (Yasumatsu *et al.*, 1998). Abecarnil and SX-3228 were more potent in displacing [^{3}H]flumazenil binding to membranes from rat cerebellum than from spinal cord or hippocampus, indicating selectivity for the BZ-ω_1 receptor subtype. These latter results are consistent to a large extent with those of previous binding studies which showed that abecarnil and SX-3228 had 5- and 7.4-fold higher affinities to cerebellar membranes compared to spinal cord membranes, respectively (Pribilla *et al.*, 1993; Ohta, 1996). Although RWJ 46771 displayed high affinities for all $GABA_A$ receptor subtypes studied, it recognized preferentially the BZ-ω_1 receptor subtype.

The *in vivo* experiments showed that all compounds increased the latency to clonic seizures produced by isoniazid. Diazepam, clobazam, abecarnil, RWJ 46771 and SX-3228 differed from bretazenil, imidazenil and Y-23684 as to the maximal increase in latency. While average increase in this measure barely reached 40 min with the latter drugs, maximal delay in onset of isoniazid-induced convulsions ranged from 60 (SX-3228) to 85 (abecarnil) min with the former. Isoniazid inhibits glutamic acid decarboxylase, the enzyme that catalyses the synthesis of GABA from glutamic acid, thereby reducing the neuronal stores of GABA available for nerve impulse-mediated release of this transmitter (Löscher and Frey, 1977). 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, and it has been proposed as an *in vivo* measure of the intrinsic activity of BZ-ω receptor ligands at $GABA_A$ receptors (Mao *et al.*, 1975). The present results are thus in agreement with electrophysiological studies showing that diazepam has greater intrinsic activity at $GABA_A$ receptors than bretazenil and imidazenil (Giusti *et al.*, 1993). However, the present data somewhat differ from those obtained by Serra *et al.* (1994b) who showed that imidazenil has a greater maximal effect than abecarnil and bretazenil on convulsions induced by isoniazid. The reasons for this discrepancy are unclear, but may include methodological factors such as the dose of isoniazid used (200 vs 800 mg/kg in the current study) or the type of convulsion measured (tonic-clonic vs clonic seizures in the present study). The present data indicate that Y-23684 may display similar intrinsic activity to bretazenil and imidazenil. This idea is strengthened by electrophysiological data showing that the enhancement of the GABA response in spinal ventral horn neurones by Y-23684 was about one-third of that by diazepam, indicating that Y-23684 possesses a lower intrinsic efficacy than diazepam on the GABA-chloride channel complex

(Yakushiji *et al.*, 1993). Results obtained with RWJ 46771 and SX-3228 suggest that they may show comparable intrinsic activity than diazepam. This proposal is consistent with *in vitro* data showing that RWJ 46771 produced a GABA shift value (1.6, from which one can assess the intrinsic activity for BZ-ω receptor ligands) somewhat greater than that observed with BZ-ω receptor partial agonists (i.e. 1.0) and close to that of the BZ-ω receptor full agonist lorazepam (i.e. 1.7) (Maryanoff *et al.*, 1995). Results with abecarnil suggest that this β-carboline may have similar or even greater intrinsic efficacy at $GABA_A$ receptors than diazepam. In line with this idea are results from electrophysiological data showing that abecarnil potentiated the GABA-induced chloride flux through α_1- and α_3-containing receptors with greater efficacies than diazepam (Pribilla *et al.*, 1993).

In the punished drinking and the lever-pressing tests in rats, compounds which have either full (diazepam, clobazam) or partial (bretazenil, imidazenil, Y-23684) agonist activity at BZ-ω receptor, without selectivity for different receptor subtypes, clearly increased rates of responding suppressed by punishment (present results, Sanger, 1995). The lack of significant modification of unpunished responding indicated that these effects were specific. It is important to note that the novel BZ-ω receptor partial agonists Y-23684 and, to a lesser extent imidazenil, displayed an efficacy comparable to those of the reference compounds in both conflict procedures. Overall, the behavioural profiles displayed by imidazenil and Y-23684 in these models are consistent with those of previous experiments in which these compounds exhibited anticonflict activity in rats over a wide dose range in the absence of effects on unpunished behaviour (Giusti *et al.*, 1993; Yasumatsu *et al.*, 1994).

The results obtained with the selective BZ-ω_1 receptor agonists in the punished drinking test showed that abecarnil and RWJ 46771 displayed anticonflict activity. SX-3228 which was tested previously under identical test conditions also showed positive effects in this test at 3 mg/kg (Griebel *et al.*, 1998). In contrast, RWJ 46771 failed to modify punished responding in the lever-pressing test. This profile is consistent with the weak efficacy of abecarnil and SX-3228 reported in previous studies using the same multiple schedule of punished and unpunished lever pressing (Sanger, 1995; Griebel *et al.*, 1998). Importantly, the anticonflict activity observed with the BZ-ω_1 receptor agonists in the punished drinking test may have been contaminated by behavioural suppression as positive effects were observed at doses which impaired unpunished responding in the lever-pressing procedure and motor performances in the actimeter and the rotarod tests. One can assume that in the punished drinking test motor deficits interfer less with responding than in the lever-pressing model so that anticonflict effects are still detectable. Taken together, the findings from conflict procedures indicate that the novel non-selective BZ-ω receptor partial agonist imidazenil and Y-23684 produced anxiolytic-like effects comparable to those of the reference compounds diazepam, clobazam and bretazenil, whereas the BZ-ω_1 receptor ligands displayed weaker and/or non-specific anxiolytic-like effects.

In the elevated plus-maze test in rats, all non-selective BZ-ω receptor agonists showed anxiolytic-like activity on all behavioural measures. These effects occurred at doses which did not decrease closed arm entries (a reliable measure of locomotor activity), thereby suggesting that the anxiolytic-like activity has not been contaminated by motor impairment. Overall, the magnitude of the positive effects of imidazenil and Y-23684 was similar to that of the reference compounds. Among the BZ-ω_1 receptor ligands, only abecarnil displayed anxiolytic-like activity comparable to that of the non-selective compounds. RWJ 46771 failed to modify significantly both spatial and temporal indices of anxiety and only weakly affected directed exploration (head-dips). However, RWJ 46771 produced a dose-related decreased in risk assessment (attempts), thereby confirming that this measure is more sensitive to drug action than are the classical elevated plus-maze measures (e.g. Cole and Rodgers, 1993; Griebel *et al.*, 1997). SX-3228 which was tested previously under identical test conditions also showed limited positive effects as it modified anxiety measures at doses (0.3–1 mg/kg) which also impaired locomotor activity (present results, Griebel *et al.*, 1998). It has been shown that, in addition to its selectivity for $GABA_A$ receptors containing the α_1-subunit on which it acts as a full agonist, abecarnil also acts as a full agonist on receptors containing the α_3-subunit but as a partial agonist at receptors containing the α_2- or α_5-subunits (Knoflach *et al.*, 1993; Pribilla *et al.*, 1993). Thus, it is conceivable that the different behavioural profile of this β-carboline in the elevated plus-maze compared to RWJ 46771 and SX-3228 might be due to an interaction with specific receptor subtypes and/or its different intrinsic activity at these sites.

Central depressant effects of traditional BZs generally seen as sedation, ataxia or myorelaxation are often manifested at anxiolytic doses. The present findings with diazepam and clobazam are in agreement with this idea as they showed that both BZs

displayed anxiolytic-like activity at doses close to those decreasing motor performances in the activity tests (see Table 3). Also consonant with the view that non-selective BZ-ω receptor partial agonists have a lower liability to produce motor impairment while retaining their anxiolytic potential, the present results with bretazenil and Y-23684 confirm that these drugs display anxiolytic-like effects at doses much lower than those producing deficits in motor performance (Martin *et al.*, 1988; Yasumatsu *et al.*, 1994). Surprisingly, imidazenil produced central depressant effects at doses which were very close to those eliciting anxiolytic-like activity. It is noteworthy that the significant decrease in motor performances induced by imidazenil contrasts with the lack of effect on the presumed motor activity measures in the elevated plus-maze (closed arm entries) (except at the highest dose of 3 mg/kg) and punished lever-pressing (unpunished responding) tests. This indicates that responses to threatening stimuli (i.e. open spaces, electric shocks) may involve central mechanisms that can override the hypolocomotor effects seen in the activity tests where there is no discrete threat stimulus and levels of anxiety are undoubtedly lower. In agreement with this idea are findings from previous studies showing that imidazenil increased exploratory activity when animals were exposed to anxiety tests (elevated plus-maze and free-exploration tests) at doses similar to those producing hypolocomotor effects in separate activity cages (Griebel *et al.*, 1996a, b). The present results with imidazenil differ from those obtained in a previous study where the drug given intravenously neither decreased locomotor activity nor produced ataxia up to 60 mmol/kg (24 mg/kg), while producing anticonflict activity at a dose 20 times lower (Giusti *et al.*, 1993). Importantly, in this study diazepam produced anxiolytic-like effects at doses (ED_{50} = 0.5 mg/kg, i.v.) which were close to those impairing motor performances (ED_{50} = 0.7 mg/kg, i.v.). Although this discrepancy might be due at least in part to the different administration routes employed (intraperitoneal vs intravenous), it is clear that more work needs to be done on the effects of imidazenil on motor activity.

As mentioned above, the BZ-ω_1 receptor ligands impaired motor performances at doses similar or close to those producing positive effects in the anxiety tests. However, the overall profile of central-depressant effects of these compounds was quite different from that displayed by the non-selective BZ-ω receptor agonists. Thus, abecarnil, RWJ 46771 and SX-3228 induced myorelaxation at doses which were 3–10 times higher than those needed to produce decrease in exploratory activity. Similar low propensity to produce muscle relaxation has been found with abecarnil in previous studies (Stephens *et al.*, 1990; Turski *et al.*, 1990) and has been described with other selective BZ-ω_1 receptor ligands such as zolpidem and alpidem (Perrault *et al.*, 1990; Zivkovic *et al.*, 1988, 1990). The present results thus confirm that drugs with selectivity for the BZ-ω_1 receptor subtype produce less muscle relaxation, and suggest further that the BZ-ω_2 subtype plays a particularly important role in mediating myorelaxant effects of BZ-ω receptor agonists (for review, see Sanger *et al.*, 1994).

In summary, the present study showed that the novel non-selective BZ-ω receptor partial agonists imidazenil and Y-23684 displayed comparable anxiolytic-like activity in rats to clinically active BZs and the partial agonist bretazenil. As bretazenil, Y-23684, but not imidazenil, showed a wide separation

TABLE 3. Minimal effective dose (MED) for anxiolytic-like activity and central depressant effects calculated from dose–response functions

	MED (mg/kg, i.p.)					
	Anxiolytic effects					
	Elevated plus-maze test	Punished lever pressing	Punished drinking test	Sedative effects Actimeter	Ataxic effects Rotarod	Myorelaxant effects Loaded grid
Diazepam	3	2.5	10	3	10	3
Clobazam	3	30	10	30	30	10
Bretazenil	0.03	1[a]	1	60	10	100
Abecarnil	0.3	>1[a]	3	1	1	10
Imidazenil	0.1	1	0.3	0.3	0.1	0.1
Y-23684	1	3	3	30	100	>60
RWJ 46771	0.1[b]	>0.3	1	0.3	0.3	1
SX-3228	0.3[c]	0.3[c]	1[c]	0.3	1	3

[a]From Sanger (1995). [b]Weak effects. [c] From Griebel *et al.* (1998).

between doses which gave rise to motor deficits and those eliciting anxiolytic-like effects, this indicated that this pyridazinone derivative may represent a valid alternative to agents currently used for the treament of anxiety disorders. Compounds with greater affinity for the BZ-ω_1 receptor subtype combined with high intrinsic efficacy displayed either weak or no anxiolytic-like effects, depending on the test used, or were active at doses which also impaired locomotor activity.

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