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Pharmacologic profile of a new anxiolytic, DN–2327: effect of Ro15–1788 and interaction with diazepam in rodents

Takeo Wada and Naohisa Fukuda

Biology Research Laboratories, Research and Development Division, Takeda Chemical Industries, Ltd., 17–85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, Japan

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Abstract. In order to characterize the pharmacologic profile of DN–2327, an isoindoline benzodiazepine (BZD) receptor ligand, its interactions with Ro15–1788 and diazepam were analyzed in rodents. The anti-conflict action of DN–2327 in two conflict tests using rats, the punished water-lick conflict (Vogel conflict) and the punished bar-pressing conflict test, was completely attenuated by treatment with Ro15–1788. The anti-convulsive (pentylenetetrazol [PTZ] induced convulsion) effect of DN–2327 was also reduced by Ro15–1788. These results suggest that the anti-conflict and anti-convulsive actions of DN–2327 may be mediated via BZD receptors. On the other hand, DN–2327 only slightly affected the motor coordination in mice and rats, as estimated by the inclined screen test and the climbing test, respectively; however, the compound attenuated the motor incoordination produced by diazepam. Furthermore, the pentobarbital potentiating effect of diazepam was reduced by pretreatment with DN–2327 in mice. In the Vogel conflict test, additive effects were observed upon the concomitant administration of subeffective doses (5 mg/kg, PO) of DN–2327 and diazepam. DN–2327 at 20 mg/kg, PO, did not reduce but slightly potentiated the anti-conflict effect of the maximum effective dose of diazepam. For PTZ-induced convulsions, DN–2327, 0.5 and 20 mg/kg, PO, doses which produced partial and complete anti-convulsive effects, respectively, in rats did not reduce but increased additively the effects of diazepam. DN–2327 at 10 and 20 mg/kg, PO, doses which both produced partial anti-convulsive effects in mice, showed an additive effect with the partial effects of diazepam. Thus, DN–2327 differentially influenced the BZD receptors: it acted as an agonist with respect to the anti-conflict and anti-convulsive effects but as an antagonist with respect to the muscle relaxant and sedative effects of diazepam. These results suggest that DN–2327 possesses receptor agonist and antagonist properties for BZD receptors.

Offprint requests to: T. Wada

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The development of benzodiazepine (BZD) receptor binding techniques (Mohler and Okada 1977) has led to the discovery of several nonBZD drugs that have apparent anxiolytic activity but reduced sedative and muscle relaxant effect (Lippa et al. 1979; Yokoyama et al. 1982; Barone et al. 1984; Petersen et al. 1984). We have reported recently that DN–2327, a novel isoindoline derivative, produced anti-conflict, taming and anti-convulsive effects when administered orally to several species of animals (Wada et al. 1989). In the Vogel conflict test and punished lever pressing procedure using rats, the minimum effective doses of DN–2327 were 10 and 2.5 mg/kg, PO, respectively, which were equipotent to those of diazepam. For pentylenetetrazol (PTZ)-induced convulsions in rats, the anti-convulsive effect of DN–2327 was much more potent ($ED_{50} = 0.95$ mg/kg, PO) than that of diazepam ($ED_{50} = 6.1$ mg/kg, PO). DN–2327 produced few of the sedative-hypnotic and muscle-relaxant effects observed with diazepam even at 160 mg/kg, PO, in rats and mice. The durations of the anti-conflict and anti-convulsive activities of DN–2327 were much longer than those with diazepam. Tolerance to DN–2327 did not develop when it was administered daily for 14 days in an anti-conflict test (Vogel conflict test). DN–2327 potently displaced [^{3}H] diazepam from BZD receptors, and the binding affinity of DN–2327 for these receptors was about 20 times that of diazepam. Furthermore, the affinity of DN–2327 for BZD receptors was not enhanced by the presence of GABA. There is a wide margin between the doses of DN–2327 that cause anti-conflict effects and those causing sedative-hypnotic/muscle-relaxant effects.

The pharmacologic profile of DN–2327 resembles those of CGS9896 (a pyrazoloquinoline), ZK91296 (a β-carboline) and CL218872 (a triazolopyridazine), etc.

Extensive studies of these compounds suggest that although they have anti-conflict effects like full agonists, they are lacking in and antagonize sedative/myorelaxant effects of full agonists (Stephens et al. 1984; Bernard et al. 1985; Cooper et al. 1987; Petersen 1987; Gardner 1988). Wood et al. (1984) proposed the name mixed agonist/antagonist for compounds which possess full agonist, pure antagonist or partial agonist activities in different assay systems.

In the present study, we examined the possibilities that the anti-conflict and anti-convulsive actions of DN–2327 are mediated via BZD receptors and that DN–2327 is a mixed agonist/antagonist by studying the effects of DN–2327 on diazepam-induced muscle relaxation and sedation. These results will help us further characterize the pharmacological profile of DN–2327.

Materials and methods

Anti-conflict activity

Vogel conflict test. Male rats (Jcl : Wistar, 151–202 g at test) were used. The experimental procedures were similar to those described by Vogel et al. (1971). The test box (light compartment: 10 × 10 × 10 cm) was enclosed in a soundproof box and equipped with a grid floor of stainless steel and a drinking bottle containing water. Rats were deprived of water for 48 h before the test. After the first 24 h of water deprivation, the rats were individually placed in the test box and given water ad libitum without any electric shock for 30 s. After another 24 h of water deprivation, the rats were again placed in the test box. DN–2327 was administered orally 1 h before and Ro15–1788 was administered intraperitoneally 30 min before the test. Diazepam was administered orally 1 h before the test in concomitant administration with Ro15–1788 and 30 min before the test in concomitant administration with DN–2327, respectively. Upon placement into the test box, each animal was allowed to drink water and to complete 20 licks or 2 s of licking before an electric current (1.5 mA, 2.5 Hz, 250 ms duration pulse shock, 2 s, electric shocker: ES–2010, Seiko Denki) was sent between the grid floor and the drinking spout through the rat. The rat received an electric shock only when it touched the drinking spout. During the subsequent 3 min test period, shocks were delivered every 20th lick or 2 s of licking. The number of shocks tolerated during the 3-min period was recorded, and these schedules were controlled by a PDP 11 microcomputer (DEC). In order to study the effects of the drugs on general water consumption, the number of licks was measured in the same way as mentioned above without any electric shocks.

Punished lever pressing procedure. The procedure was based on that described by Geller and Seifter (1960). Male rats (Jcl : Wistar, 41–51 weeks old, 246–268 g at test) were used. They were partially deprived of food and maintained at a body weight of about 250 g. The rats were trained in an operant chamber to press a lever for a milk reward (9% powdered milk solution with 1% sugar added) on a multiple variable interval of 30 s/fixed ratio 5 (VI30 s/FR5) schedule. The two schedule components were alternated during the 30-min session. Each session began with a 6 min "safety period", in which the rats were given a milk reward on a VI30 (s) schedule without any electric shock, followed by a 4-min "conflict period" signaled by a tone as a cue, in which every fifth response was reinforced with milk but also accompanied by an electric shock delivered from the grid floor (0.2 mA, 10 Hz, 0.2 s). DN–2327 and diazepam were administered orally 1 h before the test. Ro15–1788 was given intraperitoneally 10 min before the test. The effects of the drugs and saline were studied on every Tuesday and Friday, and before the test day, the rats were trained on the same schedule as the test. The twenty-four rats used were divided into six subgroups and tested on a Latin square schedule in order to counterbalance the effects of the order of drug administration.

Anti-convulsion (PTZ-induced convulsions)

Male rats (Jcl : Wistar, 6 weeks old, 131–164 g) or mice (Jcl : ICR, 4 weeks old, 20.0–26.7 g) pretreated with the test agent were given an injection of 150 mg/kg, SC, pentylenetetrazol-HCl (PTZ). The percentage of rats or mice which survived for 60 min without tonic extensor convulsions following a PTZ challenge was used to compare the anti-convulsive effects of the test agents. DN–2327 and diazepam were administered orally 1 h before and Ro15–1788 was administered intraperitoneally 30 min before the PTZ challenge in rats. In mice, all drugs were administered orally or intraperitoneally 30 min before the PTZ challenge. However, when DN–2327 was administered concomitantly with diazepam, DN–2327 was given 60 min before the PTZ challenge.

Muscle relaxation

Inclined screen test in mice. Male mice (Jcl : ICR, 4 weeks old, 16–32 g) were placed on a screen (a tetrongauze covered plate, 34 × 12 cm) which had been set at a 60 degree incline before and 0.5, 1, 1.5 and 2 h after oral administration of diazepam. DN–2327 was given orally 30 min before diazepam administration. The percentage of the mice sliding off the screen within 1 min was used to compare the muscle relaxant effects of the test agents.

Climbing test in rats. The apparatus was a piece of stainless steel wire gauze (wire size: 1.2 mm, mesh size: 1.2 × 1.2 cm, gauze: 90 cm high × 20 cm wide) placed in a vertical position 50 cm above the floor and 2 cm away from the wall. Male rats (Jcl : Wistar, 6 weeks old, 108–150 g) were placed gently 25 cm from the lower edge of the apparatus facing the upper edge 1 h and 30 min after oral administration of DN–2327 and diazepam, respectively. The percentage of the rats falling off the apparatus without reaching the upper edge within 1 min was used to compare the muscle relaxant effects of the test agents.

Sedation

Pentobarbital potentiation. A non-hypnotic dose of pentobarbital Na (25 mg/kg) was given intraperitoneally to mice (Jcl : ICR, male, 4 weeks old, 21–26 g) 1 h and 30 min after oral administration of DN–2327 and diazepam, respectively. Thereafter, mice showing loss of the righting reflex for more than 3 min within 30 min of receiving the pentobarbital were counted.

Drugs

The following drugs were used: DN–2327 (2-(7-chloro-1,8-naphthyridin-2-yl)-3-[(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-carbonylmethyl] isoindolin-1-one, Takeda) (Wada et al. 1989); diazepam (Takeda); Ro15–1788 (Flumazenil, Hoffmann-La Roche); pentylenetetrazol HCl (Sigma); pentobarbital Na (Nakarai Chemicals). DN–2327, diazepam and Ro15–1788 were suspended in 5% arabic gum saline. Pentylenetetrazol HCl and pentobarbital Na were dissolved in saline. Administration volume for rats and mice was 2 and 20 ml/kg, respectively. In the present study, the dose of Ro15–1788 was 20 mg/kg, IP, which is higher than that reported in other experiments (e.g. Quintero et al. 1985; Koob et al. 1986; Sanger 1986). This dose was chosen because the anti-convulsive effect of diazepam given at approximately an 80% effective dose was found not to be antagonized significantly by less than 20 mg/kg Ro15–1788 (data not shown).

Statistical analysis

ANOVA and W.G. Cochran's approximate *t*-test were used to evaluate the results of the Vogel conflict test and Newman–Keuls test was used for the punished lever pressing procedure. The chi-squared test was used for the anti-PTZ convulsion, pentobarbital potentiation, inclined screen and climbing tests. Unless otherwise specified, ED_{50} values were determined by Finney's probit analysis (Finney 1944).

Results

Effect of Ro15–1788 on anti-conflict and anti-convulsive activity of DN–2327

Vogel conflict test. As shown in Fig. 1, DN–2327 and diazepam at 15 mg/kg, PO, produced significant anti-conflict activity when compared to the saline control ($t = 3.89$ and 3.83, both $P < 0.01$). The anti-conflict activities of DN–2327 and diazepam were equipotent at the test dose (15 mg/kg, PO). However, in the presence of Ro15–1788, 20 mg/kg, IP, they both were antagonized to the saline control level ($t = 3.65$ and 3.63, both $P < 0.01$). Ro15–1788 alone produced no significant alteration in the number of shocks received and no behavioral changes. The time to the rat's receiving the first shock was not affected by the above drugs [$F(5,114) = 1.03$, $P > 0.1$].

In order to determine whether increases in punished drinking reflect a general increase in water consumption or a specific anti-conflict effect of DN–2327, the effect of DN–2327 on the rate of unpunished drinking was studied and compared with that of diazepam. The number of licks was slightly but not significantly increased by DN–2327 [$F(3,39) = 1.27$, $P > 0.2$] and diazepam [$F(3,38) = 1.51$, $P > 0.2$] at the doses of 5, 10 and 20 mg/kg, PO, respectively.

Punished lever pressing procedure. As shown in Fig. 2, the anti-conflict effects of DN–2327 and diazepam were equipotent at 5 mg/kg, PO ($P < 0.01$); however, DN–2327 did not affect the lever-pressing activity in the safety period, while diazepam significantly increased it ($P < 0.01$). Ro15–1788, 20 mg/kg, IP, alone had no effect on the lever-pressing activity in either the safety or conflict period; however, it antagonized the anti-conflict effect of DN–2327 as potently as it antagonized that of diazepam (both $P < 0.01$). Moreover, Ro15–1788 antagonized the increased lever-pressing activity induced by diazepam in the safety period ($P < 0.01$).

Anti-convulsion (PTZ-induced convulsions). As shown in Fig. 3, the rats receiving DN–2327, 2 mg/kg, PO, and those receiving diazepam, 20 mg/kg, PO, showed survival rates of 90% (18 of 20 rats) and 85% (17 of 20 rats), respectively, following an injection of 150 mg/kg of the convulsant PTZ. In the presence of Ro15–1788, 20 mg/kg, IP, the anti-convulsive effects of both DN–2327 and diazepam were significantly reduced: survival rates were 30% (3 of 10 rats) and 40% (4 of 10 rats), respectively (both $P < 0.01$, chi-squared test).

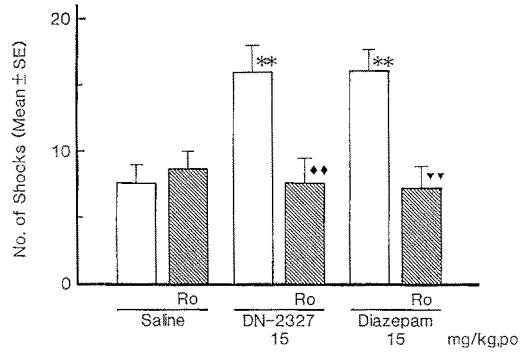

Fig. 1. Effect of Ro15–1788 on the anti-conflict action of DN–2327 and diazepam in the Vogel conflict test in rats. DN–2327 (15 mg/kg) and diazepam (15 mg/kg) were administered orally 60 min before the test. Ro15–1788 (Ro, 20 mg/kg) was administered intraperitoneally 30 min before the test. $N = 23$–36. **, ♦♦,▼▼ $P < 0.01$ versus saline-, DN–2327- and diazepam-treated groups, respectively (ANOVA and W.G. Cochran's approximate *t*-test)

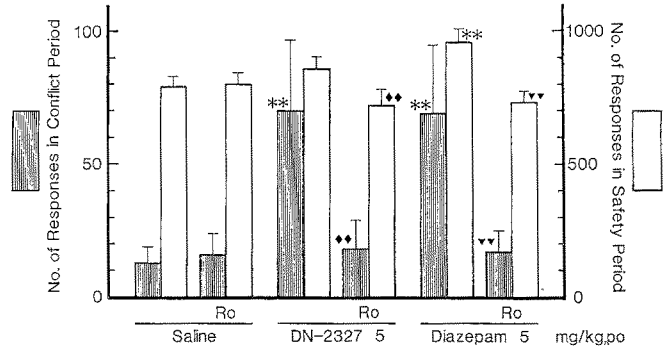

Fig. 2. Effect of Ro15–1788 on the anti-conflict action of DN–2327 and diazepam in the punished lever pressing procedure in rats. *Hatched* and *open* columns represent the number of responses in the conflict and the safety period, respectively. DN–2327 (5 mg/kg) and diazepam (5 mg/kg) were administered orally 60 min before the test. Ro15–1788 (Ro, 20 mg/kg) was administered intraperitoneally 10 min before the test. $N = 24$. **, ♦♦,▼▼ $P < 0.01$ versus saline-, DN–2327- and diazepam-treated groups, respectively (ANOVA and Newman-Keuls test)

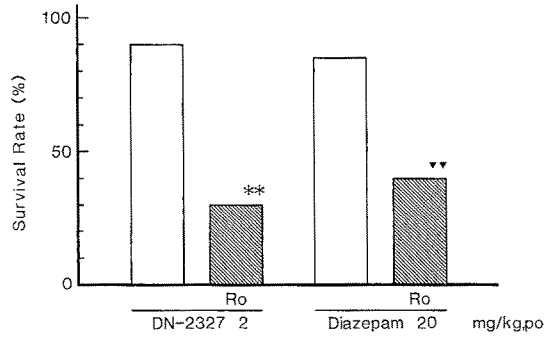

Fig. 3. Effect of Ro15–1788 on anti-convulsive (PTZ-induced) action of DN–2327 and diazepam in rats. DN–2327 (2 mg/kg) and diazepam (20 mg/kg) were administered orally 60 min before the PTZ (150 mg/kg, SC) challenge. Ro15–1788 (Ro, 20 mg/kg) was administered intraperitoneally 30 min before the PTZ challenge. $N = 10$–20. **,▼▼ $P < 0.01$ versus DN–2327- and diazepam-treated groups, respectively (chi-squared test)

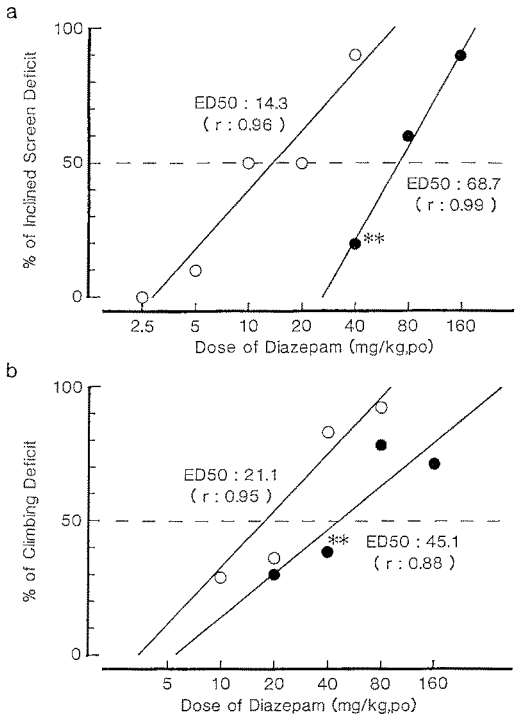

Fig. 4a, b. Dose-effect curves for diazepam administered alone and concomitantly with DN–2327 in the inclined screen test using mice **(a)** and the climbing test using rats **(b).** DN–2327 and diazepam were administered orally 60 min and 30 min before the test, respectively. DN–2327 (20 mg/kg) alone did not produce any apparent muscle relaxation. **(a)** $N=10$, **(b)** $N=7$–18. ** $P<0.01$ vs diazepam (40 mg/kg, PO)-treated group (chi-squared test). ○ Saline + diazepam; ● DN–2327 (20 mg/kg, PO) + diazepam

Interaction of DN–2327 and diazepam

Inclined screen test in mice. As shown in Fig. 4a, diazepam induced muscle relaxation dose-dependently between 2.5 and 40 mg/kg, PO, with an ED_{50} value of 14.3 mg/kg, PO (9.4–23.5 mg/kg, PO, 95% confidence limit), in the inclined screen test using mice. DN–2327, 20 mg/kg, PO, which had no effect on performance by itself, produced a parallel shift to the right in the diazepam dose-effect curve (ED_{50} value of 68.7 mg/kg, PO (41.5–101.4 mg/kg, PO, 95% confidence limit)). It significantly reduced the muscle relaxation induced by 40 mg/kg, PO, of diazepam ($P<0.01$, chi-squared test).

Climbing test in rats. As shown in Fig. 4b, diazepam induced muscle relaxation dose-dependently between 10 and 80 mg/kg, PO, with an ED_{50} value of 21.1 mg/kg, PO (11.5–29.6 mg/kg, PO, 95% confidence limit), in the climbing test using rats. However, DN–2327, 20 mg/kg, PO, which had no effect on performance by itself, produced a parallel shift to the right in the diazepam dose-effect curve [ED_{50} value of 45.1 mg/kg, PO (18.5–92.8 mg/kg, PO, 95% confidence limit)]. It significantly reduced the muscle relaxation induced by 40 mg/kg, PO, diazepam ($P<0.01$, chi-squared test).

Effect of DN–2327 on diazepam-induced pentobarbital potentiation. Diazepam caused pentobarbital potentia-

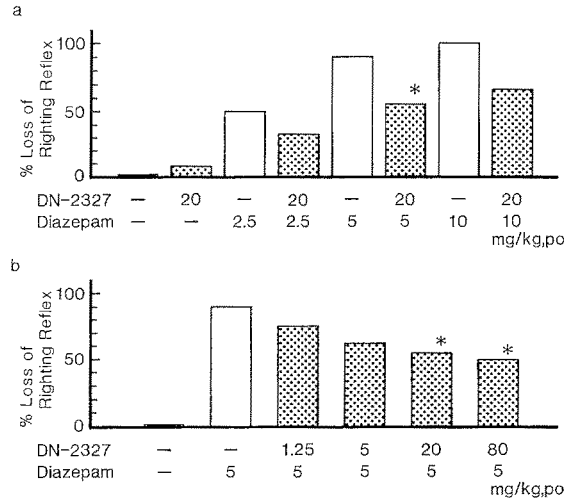

Fig. 5a, b. Antagonism of the pentobarbital potentiating effect of diazepam by DN–2327 **(a)** and the dose-dependent antagonistic effect of DN–2327 on diazepam-induced pentobarbital potentiation **(b)** in mice. DN–2327 and diazepam were given orally 60 min and 30 min, respectively before pentobarbital administration (25 mg/kg, IP). $N=8$–20. * $P<0.05$ vs diazepam (5 mg/kg, PO)-treated group (chi-squared test)

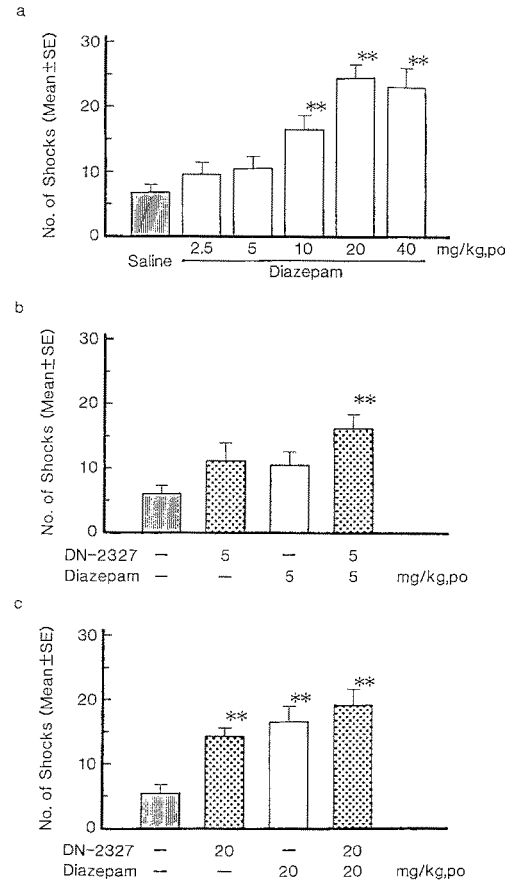

Fig. 6a–c. Dose-effect relationships for diazepam administered alone **(a),** for the concomitant administration of subeffective doses of DN–2327 and diazepam **(b)** and for the maximum effective dose of diazepam administered concomitantly with DN–2327 **(c)** in the Vogel conflict test in rats. DN–2327 and diazepam were given orally 60 min and 30 min before the test, respectively. $N=10$–24. ** $P<0.01$ vs the saline control (ANOVA and W.G. Cochran's approximate t-test)

Table 1. Anti-pentylenetetrazol (PTZ) convulsive actions of DN–2327 compared with those of diazepam (a) and the effects of DN–2327 on the anti-convulsive actions of diazepam (b) in rats and mice

(a) Anti-PTZ convulsive actions

Drug		ED_{50} value for protection against death induced by PTZ (95% confidential limits)	
		Rat[a]	Mouse
DN–2327	PO	0.95 mg/kg (0.34–1.7)	18.0 mg/kg (8.9–28.0)
	IP	0.41 (0.25–0.64)	4.2 (2.8–6.4)
Diazepam	PO	6.1 (3.7–10.9)	1.8 (1.1–3.4)

PTZ: 150 mg/kg, SC
[a] Wada et al. (1989)

(b) Effects of DN–2327 on the anti-convulsive actions of diazepam

Rat

		Saline	DN–2327 0.5 mg/kg, PO	DN–2327 20 mg/kg, PO
Saline		0/6	1/6	6/6
Diazepam	2 mg/kg, PO	1/6	3/6	6/6
	4 mg/kg, PO	2/6	3/6	6/6
	8 mg/kg, PO	3/6	5/6	6/6
	16 mg/kg, PO	6/6	6/6	6/6

Mouse

		Saline	DN–2327 10 mg/kg, PO	DN–2327 20 mg/kg, PO
Saline		0/8	3/8	4/8
Diazepam	1 mg/kg, PO	3/8	7/8	8/8
	5 mg/kg, PO	7/8	8/8	8/8

No. surviving/no. of animals used

tion dose dependently between 2.5 and 10 mg/kg, PO, in mice. DN–2327, 20 mg/kg, PO, which had little effect on pentobarbital's action by itself, suppressed the effect of each dose of diazepam. It significantly suppressed the effect of 5 mg/kg, PO, diazepam ($P<0.05$, chi-squared test) (Fig. 5a). Moreover, DN–2327 suppressed the pentobarbital potentiating effect of diazepam 5 mg/kg, PO, dose-dependently between 1.25 and 80 mg/kg, PO, and it did significantly at more than 20 mg/kg, PO ($P<0.05$, chi-squared test) (Fig. 5b).

Effect of DN–2327 on the anti-conflict activity of diazepam. In the Vogel conflict test, animals receiving diazepam at 2.5, 5, 10, 20 and 40 mg/kg, PO, tolerated a number of shocks dose-dependently [$F(5,108) = 15.70$, $P<0.01$], but the maximum effective dose was 20 mg/kg, PO (Fig. 6a).

DN–2327 at 5 mg/kg, PO, which had a sub-anticonflict effect ($t=1.75$, $0.05<P<0.01$, versus saline control) when given with diazepam at the same dose ($t=1.44$, $0.1<P<0.2$, versus saline control), additively increased the anti-conflict activity of diazepam ($t=3.47$, $P<0.01$, versus saline control) (Fig. 6b).

Although the anti-conflict effects of DN–2327 and diazepam are equipotent in the Vogel conflict test (Wada et al. 1989), the sedative/myorelaxant effects of diazepam were antagonized by 20 mg/kg, PO, DN–2327. We therefore checked to see whether or not 20 mg/kg, PO, DN–2327 had any influence on the anti-conflict activity of the maximum effective dose of diazepam.

DN–2327 at 20 mg/kg, PO, which had an anti-conflict effect ($t=2.91$, $P<0.01$, versus saline control) equipotent to that of diazepam ($t=3.93$, $P<0.01$, versus saline control) at that same dose, did not reduce but tended to increase the maximum anti-conflict activity of diazepam ($t=4.67$, $P<0.01$, versus saline control) (Fig. 6c).

Effect of DN–2327 on the anti-convulsive effect of diazepam (PTZ-induced convulsions). As shown in Table 1a, DN–2327 and diazepam reduced the number of animal deaths induced by PTZ challenge in rats and

mice. The ED_{50} values for DN–2327 and diazepam were 0.95 and 6.1 mg/kg, PO, respectively in rats (pretreatment time: 1 h and 0.5 h, respectively) and 18.0 and 1.8 mg/kg, PO, respectively, in mice (pretreatment time: both 0.5 h). By intraperitoneal administration, the ED_{50} values for DN–2327 were 0.41 and 4.2 mg/kg for rats and mice, respectively. The anti-convulsive effect of DN–2327 was much stronger than that of diazepam in rats but weaker in mice.

Diazepam alone at 2, 4, 8 and 16 mg/kg, PO, dose-dependently caused a reduction in the number of deaths induced by PTZ in rats, and DN–2327 at 0.5 mg/kg, PO, which had a partial anti-convulsive effect, additively increased the anti-convulsive effect of diazepam (Table 1b).

For the same reason as in the anti-conflict effect of DN–2327, we checked to see whether or not 20 mg/kg, PO, DN–2327 had any influence on the anti-convulsive effect of diazepam. When these doses of diazepam were given concomitantly with 20 mg/kg, PO, DN–2327, the survival rate following PTZ challenge was 100%, regardless of the dose of diazepam (Table 1b).

On the other hand, when diazepam at 1 and 5 mg/kg, PO, which dose-dependently caused a reduction in the number of deaths induced by PTZ in mice, was given concomitantly with DN–2327 at 10 and 20 mg/kg, PO, which had partial and dose-dependent anti-convulsive effects, the survival rate was not reduced but additively increased (Table 1b).

Discussion

DN–2327 binds to the BZD receptor more potently than diazepam (Wada et al. 1989). DN–2327 increased punished drinking and punished lever pressing as potently as diazepam in two conflict tests. The action of DN–2327 in these two conflict test, Vogel conflict and punished lever pressing, was completely attenuated by treatment with the BZD receptor antagonist Ro15–1788. On the other hand, DN–2327 did not increase the rate of unpunished drinking significantly. That is, the increase in punished drinking which was antagonized by Ro15–1788 does not reflect a general increase in water consumption but a specific anti-conflict effect of DN–2327. The anti-convulsive action of DN–2327 was also reduced by Ro15–1788. These results suggest that the anti-conflict and anti-convulsive actions of DN–2327 are mediated via BZD receptors.

The classical BZDs are well known to have sedative-hypnotic and muscle-relaxant activities in addition to their anti-conflict and anti-convulsive activities. DN–2327 has been reported to have anti-conflict and anti-convulsive activities which are nearly equal to and more potent than those produced by diazepam in rats, respectively. DN–2327, however, has little muscle-relaxant or sedative-hypnotic action (Wada et al. 1989). Though differences in the actions of DN–2327 and diazepam are clearly shown by these findings, it is still difficult to decide whether DN–2327 does not bind to the receptor sites related to muscle-relaxant and sedative-hypnotic action, binds to these sites but as an antagonist, or binds to all types of sites as an partial agonist.

In the present experiment, DN–2327 at a dose of 20 mg/kg, PO, a dose which had no effect on the performance in the inclined screen or climbing tests using mice and rats, produced parallel shifts to the right in the diazepam dose-effect curves. Furthermore, the pentobarbital potentiating effect of diazepam in mice was significantly attenuated by pretreatment with DN–2327 at 20 mg/kg, PO, or more. These results suggest that DN–2327 binds to the receptor sites related to muscle-relaxant and sedative-hypnotic action and that it is effective in antagonizing the muscle relaxant and sedative effects of diazepam.

If DN–2327 had been used with diazepam in the anti-conflict and anti-convulsive tests, it might have acted as an antagonist or partial agonist; the agonistic effect of diazepam might have been reduced by DN–2327. However, the concomitant administration of sub-effective doses of DN–2327 and diazepam (both 5 mg/kg, PO,) showed additive anti-conflict effects in the Vogel conflict test, furthermore, 20 mg/kg, PO, DN–2327 did not reduce but tended to increase the maximum effect of 20 mg/kg, PO, diazepam.

For anti-PTZ induced convulsion, diazepam 2–16 mg/kg, PO, prevented PTZ induced convulsions dose-dependently in rats, and DN–2327 at 0.5 mg/kg, PO, which had a partial anti-convulsive effect, additively increased the anti-convulsive effect of diazepam. In the case of concomitant administration with 20 mg/kg, PO, DN–2327, diazepam administration resulted in a 100% survival rate regardless of the dose. On the other hand, in mice, when diazepam at 1 and 5 mg/kg, PO, which dose-dependently caused a reduction in the number of deaths induced by PTZ, was given concomitantly with DN–2327 at 10 and 20 mg/kg, PO, which had partial and dose-dependent anti-convulsive effects, the survival rate following PTZ challenge was not reduced but additively increased. That is, DN–2327 did not act as an antagonist nor as a partial agonist of the anti-conflict and anti-convulsive effects of diazepam. Moreover, it has already been reported that DN–2327 exhibits full anti-conflict and anti-convulsive efficacy when administered alone (Wada et al. 1989).

In conclusion, the present results suggest that DN–2327 acts as an agonist in the case of anti-conflict and anti-convulsive effects but acts as an antagonist or a weak partial agonist in the case of muscle-relaxant and sedative effects. Wood et al. (1984) suggested that partial agonists are defined as having agonist activity in all assay systems, with this activity being characterized by a "ceiling" or reduced intrinsic activity, and in contrast, that mixed agonist/antagonists, in different assay systems, may possess full agonist, pure antagonist or partial agonist activity. From a purely behavioral point of view, DN–2327 is more accurately designated a mixed agonist/antagonist, with the profile depending on the action that is assessed.

The pharmacologic profile of DN–2327 resembles those of CGS9896, ZK91296 and CL218872, etc. These compounds are all full agonists in anxiolytic tests, where-

as CGS9896 (Bennett and Petrack 1984), ZK91296 (Stephens et al. 1984) and CL218872 (Gee et al. 1983a) are reported to lack and to antagonize the sedative/myorelaxant effects of full BZD agonists. In anti-convulsion tests, CGS9896 (Bennett and Petrack 1984), ZK91296 (Stephens et al. 1984) and CL218872 (Lippa et al. 1982) are full agonists. These data clearly indicate that these agents can be agonists, partial agonists or antagonists in different test procedures. This class of BZD receptor ligands, as well as DN–2327, can therefore be defined as agonist/antagonists.

For PTZ-induced seizures, the anti-convulsive effect of DN–2327 was much stronger than that of diazepam in rats but weaker in mice. The reason is not due to the difference in absorption between DN–2327 and diazepam, since, even by intraperitoneal administration, the anti-convulsive effect of DN–2327 was much stronger than that of diazepam in rats but not in mice. The same results have been reported with suriclone (Blanchard and Julou 1983) and CGS9896 (Yokoyama et al. 1982). CGS9896 was also reported to have only weak protection against picrotoxin-induced seizures and none against seizures induced by strychnine or electroshock (Gardner 1989). DN–2327 also offered only weak protection against bicuculline-induced seizures and none against seizures induced by electroshock (Wada et al. 1989). CGS9896 as well as DN–2327 has high affinity for BZD receptors in vitro, with IC_{50} values in the nanomolar range (Yokoyama et al. 1982; Wada et al. 1989). The ability of CGS9896 and CL218872 displace [^{3}H]flunitrazepam binding was only slightly enhanced by incubation with GABA (Brown et al. 1984; Wood et al. 1984). The ability of DN–2327 to displace [^{3}H]diazepam binding was also enhanced little by incubation with GABA (Wada et al. 1989). These pharmacologic characteristics both in vivo and in vitro may also be associated with the profile of mixed agonist/antagonists.

There is a hypothesis that could successfully explain the mixed agonist/antagonist profile in behavioral tests, as it is possible that different degrees of receptor stimulation may be required for various BZD receptor-mediated effects to emerge (Petersen et al. 1984; Gardner 1989). Gardner (1989) proposed the working hypothesis that there is a continuum of behaviors related to the degree of activation of BZD receptors, extending from muscle relaxation at maximum occupancy of a full agonist, through sedative, anti-convulsant and anxiolytic effects, to antagonists with little intrinsic activity. The actions of ZK91296 have been interpreted in this fashion, with greater intrinsic efficacy required for motor impairment than for anticonvulsant activity (Klockgether et al. 1985).

The behavioral profile of a BZD receptor ligand on this continuum will depend on the total of the affinity, availability and intrinsic activity of the ligand. The bioavailability of DN–2327 has not been investigated thoroughly; however, the affinity of DN–2327 for the BZD receptor is much higher than that of a BZD full agonist, diazepam (Wada et al. 1989). In this working hypothesis, if DN–2327 is a partial agonist for BZD receptors, the present and previous data on DN–2327 (Wada et al. 1989) might be almost completely accounted for. Especially, in the test requiring a lower degree of stimulation at the BZD receptors, i.e. PTZ-induced convulsion in rats, the higher affinity of DN–2327 for the BZD receptors may account for DN–2327's effect being stronger than that of diazepam. In contrast to this, in the test requiring a higher degree of stimulation at the BZD receptors, i.e. muscle relaxation and pentobarbital potentiation, the low intrinsic activity of DN–2327 may account for DN–2327's playing the role of an antagonist or a weak partial agonist.

However, some data are inconsistent. Firstly, although the anti-convulsive effects of diazepam against PTZ- and bicuculline-induced convulsions were almost the same in rats (ED_{50}: 6.1 and 6.4, respectively), the anti-convulsive effects of DN–2327 were much stronger against PTZ- than bicuculline-induced convulsions (ED_{50}: 0.95 and 6.5, respectively) (Wada et al. 1989). Secondly, although 20 mg/kg, PO, DN–2327 antagonized the pentobarbital potentiation caused by 5 mg/kg, PO, of diazepam in mice (Fig. 5), the same dose of DN–2327 potentiated the anti-convulsive effects of 1 and 5 mg/kg, PO, diazepam in mice (Table 1).

Moreover, there are some problems with the behavioral continuum proposed by Gardner (1989). PK8165 and PK9084, quinoline derivatives, are full agonists in anxiolytic tests and weak partial agonists in sedative paradigms (File 1983); however in anti-convulsant tests, they are BZD antagonists (Gee et al. 1983b). The agents CGS9895 and RU39419, which are a pyrazoloquinoline and a hydroxyquinoline, respectively, appear to be partial agonists in that they show bell-shaped dose-response curves with low ceilings in anxiolytic, anticonvulsant and myorelaxant tests (Yokoyama et al. 1982). These data, which will need to be more precisely examined with respect to the affinity, bioavailability, and intrinsic activity of these ligands, cannot be explained by the working hypothesis, which depends on the only one type of BZD receptor.

There is another hypothesis which states that DN–2327 may have agonistic actions on some BZD receptor subtypes but antagonistic actions on others. Similar hypotheses have been proposed for CL218872, quazepam, premazepam, CGS9896 and ZK91296, all of which have been reported to have anti-conflict activity without an ataxic/muscle relaxant potential (Lippa et al. 1979; Gee and Yamamura 1982; Sieghart 1983; Barone et al. 1984; Bennet and Petrack 1984; Petersen et al. 1984; Bernard et al. 1985; Pellow and File 1986).

This hypothesis is based on the belief that the affinity or efficacy at cerebellar BZD receptors may differ from that at cortical receptors. That is, CL218872 shows a preference for the higher-affinity BZD_1 sites which account for virtually all binding in the cerebellum, but occurred in different proportions with the lower-affinity BZD_2 sites in most other brain regions (Klepner et al. 1979; Williams 1984). It was originally suggested from the profile of CL218872 that BZD_1 sites were associated with anxiolytic properties and BZD_2 with sedative/muscle relaxant properties (Klepner et al. 1979). However, there are some inconsistencies. Firstly, the cerebel-

lum which is rich in the BZD_1 type, is not usually associated with anxiolytic but with muscle-relaxant properties. Secondly, zolpidem, which is another BZD receptor ligand with selectivity for BZD_1 sites, possesses a potential CNS depressant property, that is, sedation as well as anxiolytic and anti-convulsant properties (Sanger et al. 1987). Thirdly, such binding characteristics could result from negative cooperativity of conformational changes in a receptor protein, and debate over these interpretations continues (Lippa et al. 1982; Martin et al. 1983; Sieghart 1985).

We cannot draw definite conclusions about DN–2327, because we have not yet determined whether DN–2327 binds selectively to some BZD receptor sites. Even if there are some specific BZD receptor binding sites associated with each pharmacologic profile, DN–2327 and the other agonist/antagonists may bind to all BZD receptor sites, and in the case that they do not bind as agonists, they will bind as antagonists.

Recently, as it was reported that GABA receptors have some subtypes in the CNS at the gene level (Levitan et al. 1988; Pritchett et al. 1989), biotechnological investigation may also answer the question as to whether or not there are different types of BZD receptor sites in the CNS.

Regardless of these molecular considerations, the behavioral profile of DN–2327 suggests that DN–2327 acts as an agonist like diazepam in the case of anti-conflict and anti-convulsive effects, but acts as an antagonist like Ro15–1788 or a weak partial agonist in the case of muscle-relaxant and sedative effects.

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References

Barone D, Colombo G, Glasser A, Luzzani F, Mennini T (1984) In vitro interaction of premazepam with benzodiazepine receptors in rat brain regions. Life Sci 35:365–371

Bennett DA, Petrack B (1984) CGS9896: a nonbenzodiazepine, nonsedating potential anxiolytic. Drug Dev Res 4:75–82

Bernard PS, Bennett DA, Pastor G, Yokoyama N, Liebman JM (1985) CGS9896: agonist-antagonist benzodiazepine receptor activity revealed by anxiolytic, anticonvulsant and muscle relaxation assessment in rodents. J Pharmacol Exp Ther 235:98–105

Blanchard JC, Julou L (1983) Suriclone: a new cyclopyrrolone derivative recognizing receptors labeled by benzodiazepines in rat hippocampus and cerebellum. J Neurochem 40:601–607

Brown CL, Martin IL, Jones B, Oakley N (1984) In vivo determination of efficacy of pyrazoloquinolinones at the benzodiazepine receptor. Eur J Pharmacol 103:139–143

Cooper SJ, Kirkham TC, Estall LB (1987) Pyrazoloquinolines: second generation benzodiazepine receptor ligands have heterogeneous effects. TIPS 8:180–184

File SE (1983) Sedative effects of PK9084 and PK8165, alone and in combination with chlordiazepoxide. Br J Pharmacol 79:219–223

Finney DJ (1944) The application of probit analysis to the results of mental test. Psychometrika 9:31–39

Gardner CR (1988) Pharmacological profiles in vivo of benzodiazepine receptor ligands. Drug Dev Res 12:1–28

Gardner CR (1989) Interpretation of the behavioral effects of benzodiazepine receptor ligands. Drugs of the Future 14: 51–67

Gee KW, Yamamura HI (1982) A novel pyrazoloquinoline that interacts with brain benzodiazepine receptors: characterization of some in vitro and in vivo properties of CGS9896. Life Sci 30:2245–2252

Gee KW, Brinton RE, Yamamura HI (1983a) CK218872 antagonism of diazepam-induced loss of righting reflex: evidence for partial agonistic activity at the benzodiazepine receptor. Life Sci 32:1037–1040

Gee KW, Brinton RE, Yamamura HI (1983b) PK8165 and PK9084, two quinoline derivatives with anxiolytic properties, antagonize the anticonvulsant effects of diazepam. Brain Res 264:168–172

Geller I, Seifter J (1960) The effects of meprobamate, barbiturate, *d*-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 1:382–492

Klepner CA, Lippa AS, Benson DI, Sano MC, Beer B (1979) Resolution of two biochemically and pharmacologically distinct benzodiazepine receptors. Pharmacol Biochem Behav 11:457–462

Klockgether T, Schwarz M, Turski L, Sontag KH (1985) ZK91296, an anti-convulsant β-carboline which lacks muscle relaxant properties. Eur J Pharmacol 110:309–315

Koob GF, Braestrup C, Britton KT (1986) The effects of FG7142 and Ro15–1788 on the release of punished responding produced by chlordiazepoxide and ethanol in the rat. Psychopharmacology 90:173–178

Levitan ES, Schofield PR, Burt DR, Rhee LM, Wisden W, Kohler M, Fujita N, Rodriguez HF, Stephenson A, Darlison MG, Barnard EA, Seeburg PH (1988) Structural and functional basis for GABA–A receptor heterogeneity. Nature 335:76–79

Lippa AS, Coupet J, Greenblatt EN, Klepner CA, Beer B (1979) A synthetic non-benzodiazepine ligand for benzodiazepine receptors: a probe for investigating neuronal substrates of anxiety. Pharmacol Biochem Behav 11:99–106

Lippa AS, Meyerson LR, Beer B (1982) Molecular substrates of anxiety: clues from the heterogeneity of the benzodiazepine receptors. Life Sci 31:1409–1417

Martin IL, Brown CL, Doble A (1983) Multiple benzodiazepine receptors: structures in the brain or structures in the mind? A critical review. Life Sci 32:1925–1933

Mohler M, Okada T (1977) Benzodiazepine receptor: demonstration in the central nervous system. Science 198:849–851

Pellow S, File SE (1986) Evidence that the β-carboline, ZK91296, can reduce anxiety in animals at doses well below those causing sedation. Brain Res 363:174–177

Petersen EN (1987) Benzodiazepine receptor pharmacology: new vistas. Drugs of the Future 12:1043–1053

Petersen EN, Jensen LH, Honore T, Braestrup C, Kehr W, Stephens DN, Wachtel H, Seidelman D, Schmiechen R (1984) ZK91296, a partial agonist at benzodiazepine receptors. Psychopharmacology 83:240–248

Pritchett DB, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR, Seeburg PH (1989) Importance of a novel GABA–A receptor subunit for benzodiazepine pharmacology. Nature 338:582–585

Quintero S, Henney S, Lawson P, Mellanby J, Gray JA (1985) The effects of compounds related to γ-aminobutyrate and benzodiazepine receptors on behavioural responses to anxiogenic stimuli in the rat: punished barpressing. Psychopharmacology 85:244–251

Sanger DJ (1986) Investigation of the actions of the benzodiazepine antagonists Ro15–1788 and CGS8216 using the schedule-controlled behavior of rats. Pharmacol Biochem Behav 25:537–541

Sanger DJ, Perrault G, Morel E, Joly D, Zivkovic B (1987) The behavioral profile of zolpidem, a novel hypnotic drug of imidazopyridine structure. Physiol Behav 41:235–240

Sieghart W (1983) Several new benzodiazepines selectively interact with a benzodiazepine receptor subtype. Neurosci Lett 38:73–78

Sieghart W (1985) Benzodiazepine receptors: multiple receptors or multiple conformations? J Neural Transm 63:191–208

Stephens DN, Kehr W, Schneider HH, Braestrup C (1984) Bidirectional effects on anxiety of β-carbolines acting as benzodiazepine receptor ligands. Neuropharmacology 23:879–880

Vogel JR, Beer B, Clody DE (1971) A simple and reliable conflict procedure for testing antianxiety agents. Psychopharmacologia 21:1–7

Wada T, Nakajima R, Kurihara E, Narumi S, Masuoka Y, Goto G, Saji Y, Fukuda N (1989) Pharmacologic characterization of a novel non-benzodiazepine selective anxiolytic, DN–2327. Jpn J Pharmacol 49:337–349

Williams M (1984) Molecular aspects of the action of benzodiazepine and non-benzodiazepine anxiolytics: a hypothetical allosteric model of the benzodiazepine receptor complex. Prog Neuropsychopharmacol Biol Psychiatry 8:209–247

Wood PL, Loo P, Braunwalder A, Yokoyama N, Cheney DL (1984) In vitro characterization of benzodiazepine receptor agonists, antagonists, inverse agonists and agonist/antagonists. J Pharmacol Exp Ther 231:572–576

Yokoyama N, Ritter B, Neubert A (1982) 2-Arylpyrazolo[4,3-c] quinolin-3-ones: novel agonist, partial agonist and antagonist of benzodiazepines. J Med Chem 25:337–339