Effects of Brotizolam, a New Thieno-Triazolo-Diazepine Derivative, on the Central Nervous System

Kenjiro KIMISHIMA, Kyoko TANABE, Yukako KINOSHITA, Kooji TOKUYOSHI, Daisuke HOURI and Tatsuo KOBAYASHI
Department of Pharmacology, Tottori University School of Medicine, Yonago 683, Japan

Accepted August 24, 1984

Abstract—The effects of brotizolam, a new thieno-triazolo-diazepine derivative, on the central nervous system were analyzed in mice, rats and rabbits. Diazepam, estazolam and triazolam were used as control drugs. Brotizolam inhibited spontaneous motor activities; performances in the rota rod test, staircase test, and maximal electroshock seizure test; and pentetrazol- or bemegride-induced convulsion. Moreover, catalepsy inducing action and potentiating effect on sleep elicited by pentobarbital or ethanol were observed. Following intraperitoneal or oral administration of brotizolam to rabbits with chronically implanted electrodes, the electro-encephalographic profile in spontaneous EEG was characterized by slow waves with high amplitudes in the neocortex. The arousal responses by stimulation of the midbrain reticular formation and posterior hypothalamus were slightly inhibited, but the recruiting responses induced by stimulation of the diffuse thalamic projecting system were not inhibited, and seizure discharges induced by stimulation of the dorsal hippocampus were inhibited markedly. When motor activities and pentetrazol-induced convulsions were observed as indices of tolerance for brotizolam, tolerance was not developed by repeated administration of brotizolam up to 14 days. These results suggested that brotizolam, a new thieno-triazolo-diazepine derivative, is judged to be a safer and stronger sleep inducer than diazepam and estazolam.

Since the pioneering paper by Randall et al. (1) showed that chlordiazepoxide is a new type of minor tranquilizer with sedative, muscle relaxant and anticonvulsive actions, new derivatives of benzodiazepine such as diazepam (2), nitrazepam (3), bromazepam (4) and medazepam (5) have been developed, and they have been in wide clinical use as remedies for psychoneuroses such as anxiety and psychosomatic catatonia. Among them, diazepam, nitrazepam and flurazepam (4) are nowadays used as sleep inducers, and in this field, estazolam (6) and triazolam (7), new triazolo-benzodiazepine compounds having a trizol ring, have invited interest in recent years.

On the other hand, studies of benzodiazepine analogues led to research and development of thienodiazepine compounds (8–10). New thieno-triazolo-diazepines with a triazol ring were recently developed by Weber et al. (11) and Nicholson et al. (12). Brotizolam, 2-bromo-4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]-triazolo-[4,3-a][1,4]-diazepine, has the chemical structure shown in Fig. 1. It is a white, odorless powder that is insoluble in water, soluble in chloroform, and slightly soluble in methyl alcohol.

In the present study, its actions on the central nervous system were investigated.

Materials and Methods

Animals: In this study ddY-strain mice, Wistar rats and matured rabbits were mainly used, and cats were used in part of the experiments.

General behavior: Each drug was administered orally or intraperitoneally, and general behavior was observed up to 8 hr after administration.

Measurement of spontaneous locomotor
activity: Spontaneous locomotor activity of mice of each group consisting of 6 mice was recorded up to 6 hr after administration with an Animex activity meter (Farad Electronics, Sweden) using the resonance circuit of the induction coil.

Rotarod test: Mice of each group consisting of 10 mice or more were put on a rotarod having a diameter of 3 cm and revolving at 16 r.p.m., and those which did not fall off for 3 min or more were regarded as normal.

Catalepsy test: According to Matsuda's method (13), mice were forced to lay their forelegs on a horizontal bar at 6 cm in height. Normal mice should try immediately to free themselves from the forced posture. Those which had kept the posture for 30 sec or more were judged to be catalepsy positive.

Sleep potentiating action: Male mice of each group consisting of 8 or more, which had been pre-treated with brotizolam or a control drug for comparison, were injected with pentobarbital, intraperitoneally, at a dose of 35 mg/kg or 20% ethanol, intravenously, at a dose of 0.1 ml/10 g body weight, and the drug effects on the onset time and duration of sleep were investigated.

Anticonvulsive action: Male mice of each group consisting of 10–20 animals were orally administered the test or control drug, and at 2 hr after administration, the anticonvulsive action was examined by both the maximal electroshock seizure test and drug-induced convulsions method (pentetrazol and bemegride). The maximal electroshock (50 mA, for 0.2 sec) was given to mice using the apparatus described by Woodbury and Davenport (14) through corneal electrodes, and the disappearance of tonic extensor (TE) induced by the electroshock was taken as the determinant of the anticonvulsive effect of drugs. As to chemoshock, the effects of these drugs were investigated on the minimal full (MF), tonic flexor (TF) and tonic extensor (TE) seizure induced by the subcutaneous injection of pentetrazol and bemegride at doses of 95 and 38 mg/kg, respectively.

Staircase test: This is a simple test method devised by Thiébot et al. (15) for screening of anxiolytic drugs. The wooden device used in this test is a box, 30 cm high and 95 cm long, that contains a 5 story staircase, with steps that are each 20 cm wide, 6 cm high and 15 cm long. Rats weighing about 200 g were placed quietly at the bottom of the staircase with their back toward the staircase, and then the frequencies of rearing and climbing the staircase were recorded for the subsequent 3 min.

EEG: Unanaesthetized rabbits were fixed in a stereotaxic apparatus, and bipolar electrodes of insulated stainless steel of 0.25 mm in diameter were implanted in 3 areas of the cortex (the frontal, temporal and occipital part) and 3–5 subcortical areas of the brain (the posterior hypothalamus, thalamic medial nucleus, midbrain reticular formation, dorsal hippocampus and amygdaloid nucleus). These rabbits were subjected to the test at one week after the above operation. These electrodes were connected to a pen-writing 8-lead electroencephalograph for recording EEG at a rate of 1.5 cm per sec. For the purpose of intracerebral stimulation, the same electrodes were used, and after stimulation, these electrodes were used as lead electrodes.
through turning of a switch. For stimulation, a rectangular pulse was used, parameters of which will be described in the related paragraph.

**Tolerance test:** In the tolerance-producing liability test where motor activities and pentetrazol-induced convulsions were used as indices of tolerance, brotizolam was administered to mice for two consecutive weeks.

**Statistics:** Statistical significance of the data was estimated using Student's *t*-test. The values of ED50 and the 95% confidence limit were calculated by the method of Litchfield and Wilcoxon (16),

**Drugs and administration route:** As it is insoluble in water, brotizolam was administered to animals orally or intraperitoneally as a 0.2% CMC suspension. As the active control, diazepam, estazolam or triazolam was used (Fig. 1).

**Results**

1. **General behavior**
   a) **Mice:** Following the oral administration of brotizolam at doses of 0.5 mg/kg or less, no change was observed in the general behavior of mice. At doses of 1–2 mg/kg, most of the mice kept a sedative crouching posture for 4–6 hr after administration. At dose of 5–20 mg/kg, mice were under sedation for longer periods, but recovered from the sedation by the morning of the following day.
   b) **Rabbits:** After intraperitoneal injection of brotizolam at doses of 0.5–1 mg/kg, no change was observed in the general behavior. However, at doses of 2–5 mg/kg, the spontaneous locomotor activity decreased; some rabbits kept crouching, motionless, and they somewhat dragged their hind legs when forced to move. Such symptoms lasted up to 3 hr after administration.
   c) **Cats:** In several minutes after intraperitoneal injection of brotizolam at a dose of 5 mg/kg, cats started to develop conspicuous muscular relaxation at the hind legs, showing a rather severe ataxic gait. With respect to consciousness, on the other hand, they were rather excited, biting softly and frolicking. In 30–40 min after the injection, the animals entered into sedation, but some of them still showed frolicking behavior. At 24 hr after the injection, some animals still showed a slight ataxic gait.

2. **Spontaneous locomotor activity**
   Mice of each group consisting of 6 animals were placed in plastic observation cages. After recording of their locomotor activity for 2 hr, the test or the control drug was administered, and the spontaneous locomotor activity of these animals was automatically counted, up to the subsequent 6 hr.

Brotizolam administered orally at a dose of 0.1 mg/kg did not exert any effect on the spontaneous locomotor activity of these animals. In the 0.5 mg/kg dose group, spontaneous locomotor activity was slightly inhibited from 30 min to 6 hr after administration. In the 1 mg/kg and 2 mg/kg dose groups, a marked inhibition of spontaneous locomotor activity was observed from 30 min up to over 6 hr after administration as shown in Fig. 2.

In both groups that were orally administered diazepam and estazolam at a dose of 2 mg/kg, moderate inhibition of spontaneous locomotor activity was observed...
from 1 up 6 hr after administration (Fig. 3), but the inhibition was found to be weaker than in the 1 mg/kg brotizolam group.

3. Rotarod test

The test was performed with mice which could stay on the rotarod for 3 min or more in each preliminary test, performed thrice on the day before the start of this test. The test was conducted twice at 30 min and 1 hr after administration of each drug. In both tests, the number of animals which fell off the rotarod increased at doses of 0.1 mg/kg brotizolam or more. The ED50 of brotizolam 1 hr after administration was 0.26 mg/kg (Table 1).

The ED50 of estazolam was calculated to be 0.53 mg/kg and that of triazolam, 0.66 mg/kg.

4. Catalepsy test

The test was performed with 10 mice in each group. As shown in Table 2, brotizolam caused catalepsy at an oral dose of 0.05 mg/kg, but the incidence of catalepsy was not observed to be dose-dependent. In estazolam groups, catalepsy also appeared in a dose-independent manner in one case each at 0.05 mg/kg and 0.5 mg/kg.

5. Sleep potentiating action

a) Pentobarbital-induced sleep: One hr after the oral administration of brotizolam at different doses to mice of each group consisting of 9–10 animals, pentobarbital was intraperitoneally injected at a dose of 35 mg/kg, and the onset and duration of sleep was measured. As shown in Table 3, brotizolam slightly shortened the onset time of sleep at doses of 0.1–1 mg/kg, whereas the duration of sleep was prolonged by more than two times that

![Fig. 3. Effects of diazepam and estazolam on spontaneous locomotor activities in mice. Ordinate: counts/min. Abscissa: time in hr. A: after administration of diazepam, 2 mg/kg and B: estazolam, 2 mg/kg, p.o.](image)

Table 1. Effects of brotizolam and estazolam on the rotarod test in mice

| Drug   | Dose (mg/kg, p.o.) | No. of animals used | No. of falling before | 30 min | 1 hr |
|--------|--------------------|---------------------|-----------------------|--------|------|
| Control|                    | 10                  | 0                     | 0      | 0    |
| Brotizolam | 0.05               | 10                  | 0                     | 0      | 1    |
|         | 0.1                | 10                  | 0                     | 3      | 3    |
|         | 0.2                | 10                  | 0                     | 3      | 4    |
|         | 0.5                | 20                  | 0                     | 16     | 14   |
|        | ED50=0.26 (0.14–0.47) mg/kg, p.o. |                     |                       |        |      |
| Estazolam | 0.05               | 10                  | 0                     | 0      | 1    |
|         | 0.1                | 10                  | 0                     | 3      | 2    |
|         | 0.2                | 10                  | 0                     | 0      | 2    |
|         | 0.5                | 10                  | 0                     | 5      | 4    |
|         | 1                  | 10                  | 0                     | 8      | 8    |
|        | ED50=0.53 (0.25–1.11) mg/kg, p.o. |                     |                       |        |      |
| Triazolam|                    |                     |                       |        |      |
|         | ED50=0.66 (0.28–1.48) mg/kg, p.o. |                     |                       |        |      |
observed with the control at a dose of 0.01 mg/kg, by about three times at 0.5 mg/kg, and 4 times at 1 mg/kg. In estazolam-administered mice, the onset time of sleep tended to delay, while the duration of sleep was prolonged significantly at doses of 0.05–1 mg/kg.

The ED50 of brotizolam and estazolam calculated in animals that kept sleeping for periods at least two times as long as the animals of the control group to which pentobarbital alone was administered (29 min) were 0.042 mg/kg and 0.068 mg/kg, respectively.

**Table 2. Effects of brotizolam and estazolam on the catalepsy test in mice**

| Drug     | Dose (mg/kg, p.o.) | No. of animals used | No. of catalepsy |
|----------|--------------------|---------------------|------------------|
|          |                    |                     | 1 hr  | 2 hr  |
| Control  |                    |                     | 0     | 0     |
|          | 0.01               | 10                  | 0     | 0     |
|          | 0.05               | 10                  | 0     | 0     |
| Brotizolam| 0.1               | 10                  | 0     | 0     |
|          | 0.2                | 10                  | 2     | 3     |
|          | 0.5                | 10                  | 1     | 0     |
|          | 0.05               | 10                  | 1     | 0     |
| Estazolam| 0.1                | 10                  | 0     | 0     |
|          | 0.2                | 10                  | 0     | 0     |
|          | 0.5                | 10                  | 1     | 0     |

**Table 3. Effects of brotizolam and estazolam on sleeping time induced by pentobarbital (35 mg/kg, i.p.)**

| Drug     | Dose (mg/kg, p.o.) | No. of animals used | Sleeping time (min±S.E.) |
|----------|--------------------|---------------------|--------------------------|
|          |                    |                     | onset       | duration  |
| Control  |                    | 10                  | 4.2±0.2     | 29.0±2.8  |
|          | 0.005              | 10                  | 3.7±0.2     | 45.5±5.7* |
|          | 0.01               | 9                   | 3.6±0.4     | 64.1±7.2**|
|          | 0.05               | 9                   | 5.0±0.7     | 55.3±7.8**|
| Brotizolam| 0.1               | 10                  | 3.0±0.3**   | 82.2±7.4**|
|          | 0.2                | 10                  | 3.0±0.2**   | 78.3±10.1**|
|          | 0.5                | 10                  | 3.3±0.2**   | 92.0±14.0**|
|          | 1                  | 10                  | 3.1±0.2**   | 112.4±13.4**|
| Estazolam| 0.01              | 10                  | 8.2±0.9**   | 30.3±6.5  |
|          | 0.02               | 10                  | 6.5±0.4**   | 36.9±5.6  |
|          | 0.05               | 10                  | 6.0±0.5**   | 47.4±7.4* |
|          | 0.1                | 10                  | 5.6±0.5*    | 51.8±6.9* |

*P<0.05, **P<0.01

b) Ethanol-induced sleep: One hr after brotizolam, ethanol (20 V/V %) was injected intravenously into male mice of each group consisting of 8–12 at a dose of 0.1 ml/10 g. As shown in Table 4, sleeping time was markedly prolonged in a dose-dependent manner by brotizolam, though the values showed considerable variation.

6. Anticonvulsive action

a) Maximal electroshock seizure: Two hr after the oral administration of the drugs, an electric shock was given to mice of each group composed of at least 15 mice, and the anticonvulsive effects of these drugs were examined.

As indicated in Table 5, 75 mg/kg
brotizolam inhibited the appearance of TE in half of the mice, but the rate of inhibition did not increase further even when the dose was increased to 100 mg/kg, the ED50 of brotizolam for inhibition of TE being more than 75 mg/kg.

Estazolam also inhibited TE dose dependently, the ED50 of estazolam being 41.0 mg/kg.

b) Pentetrazol-induced convulsions: Following the subcutaneous injection of pentetrazol at a dose of 95 mg/kg, mice began to develop MF at 5–15 min after injection and showed slight excitement, jumping repeatedly and moving around in the cage, and some mice developed transient clonic convulsions (CL). At 7–50 min after injection, however, almost all of the mice developed TF and subsequent TE and died. Judgement on anticonvulsive action was made on the basis of the disappearance of MF, TF or TE seizure.

The effects of the drugs on the pentetrazol-induced convulsions at 2 hr after the administration were examined.

As shown in Table 6, the appearance of pentetrazol-induced convulsions were markedly inhibited after administration of brotizolam at a dose of 0.2 mg/kg or more and estazolam at 0.5 mg/kg or more. The ED50 of brotizolam, estazolam, diazepam and triazolam were 0.27 mg/kg, 0.66 mg/kg, 0.48 mg/kg and 0.012 mg/kg, respectively.

c) Bemegride-induced convulsions: When bemegride was subcutaneously injected at a dose of 38 mg/kg (100% convulsive dose), mice developed a series of convulsions in a process almost similar to that described above for pentetrazol-induced convulsions. At this dose, all mice suffered from convulsions such as MF, TF and TE seizure and about 95% of them died immediately after TE.

At 2 hr after oral administration of the test
and control drugs, bemegride was injected subcutaneously. As shown in Table 7, the appearance of bemegride-induced convulsions were markedly inhibited after treatment with brotizolam at a dose of 0.2 mg/kg or more and estazolam at 1 mg/kg or more, the calculated ED50 values being 0.21 mg/kg and 0.71 mg/kg, respectively. The ED50 of diazepam and triazolam were 1.2 mg/kg and 0.014 mg/kg, respectively.

| Drug        | Dose (mg/kg, p.o.) | No. of animals used | No. of convulsion | No. of death |
|-------------|--------------------|---------------------|-------------------|--------------|
| Control     |                    |                     |                   |              |
| 0.05        | 11/11              | 5/5                 | 11 (100)          | 5            |
| 0.1         | 20/15              | 4/4                 | 15 (74)           | 4            |
| Brotizolam  | 0.2                | 13/4                | 13 (68)           | 4            |
| 0.3         | 20/9               | 3/3                 | 9 (46)            | 3            |
| 0.5         | 20/3               | 0/0                 | 3 (15)            | 0            |
|             |                    |                     |                   |              |
| Estazolam   | 0.1                | 18/8                | 18 (90)           | 8            |
| 0.2         | 16/2               | 2/2                 | 16 (80)           | 2            |
| 0.5         | 12/2               | 2/2                 | 12 (60)           | 2            |
| 1           | 8/0                | 0/0                 | 8 (40)            | 0            |
| 1.5         | 2/0                | 0/0                 | 2 (10)            | 0            |
| 2           | 0/0                | 0/0                 | 0/0               | 0            |
|             |                    |                     |                   |              |
| Diazepam    | ED50=0.27 (0.19–0.39) mg/kg, p.o. |                     |                   |              |
| Triazolam   | ED50=0.48 (0.30–0.60) mg/kg, p.o. |                     |                   |              |

| Drug        | Dose (mg/kg, p.o.) | No. of animals used | No. of convulsion | No. of death |
|-------------|--------------------|---------------------|-------------------|--------------|
| Control     |                    |                     |                   |              |
| 0.05        | 19/11              | 11/11               | 19 (95)           | 11           |
| 0.1         | 14/0               | 0/0                 | 14 (70)           | 0            |
| Brotizolam  | 0.2                | 11/0                | 11 (55)           | 0            |
| 0.5         | 8/0                | 0/0                 | 8 (40)            | 0            |
| 1           | 1/0                | 0/0                 | 1 (5)             | 0            |
|             |                    |                     |                   |              |
| Estazolam   | 0.1                | 20/11               | 20 (100)          | 11           |
| 0.2         | 19/11              | 11/11               | 19 (95)           | 11           |
| 0.5         | 16/8               | 8/8                 | 16 (80)           | 8            |
| 1           | 3/0                | 0/0                 | 3 (15)            | 0            |
|             |                    |                     |                   |              |
| Diazepam    | ED50=0.71 (0.51–0.98) mg/kg, p.o. |                     |                   |              |
| Triazolam   | ED50=1.2 (0.9–1.7) mg/kg, p.o. |                     |                   |              |
|             | ED50=0.014 (0.01–0.018) mg/kg, p.o. |                     |                   |              |
7. Staircase test
The effects of brotizolam and benzodiazepines on the staircase test which is generally used for screening of anxiolytic drugs were examined. Each drug was administered orally 2 hr before the test.

As shown in Fig. 4, brotizolam showed almost the same tendency as that of other benzodiazepines. Compared with the results obtained with untreated rats, brotizolam clearly augmented the frequency of climbing the staircase at doses of 0.1–2 mg/kg, but reduced the frequency at 4–8 mg/kg, while the drug reduced the frequency of rearing dose-dependently within the range of 1–4 mg/kg. After administration of estazolam also, the frequency of climbing was augmented at doses of 0.1–4 mg/kg, but reduced clearly at a dose of 8 mg/kg, while the frequency of rearing was reduced dose-dependently within the range of 1–8 mg/kg.

8. Effects on EEG
The effects of brotizolam on EEG were investigated by administering the drug orally or intraperitoneally to rabbits with chronically implanted electrodes.

a) Spontaneous EEG: At 5–10 min after administration of brotizolam at doses of 2–5 mg/kg, the fast-wave components in the cortex began to decrease and high-voltage waves appeared sometimes. The θ wave at the hippocampus became rather irregular when the drug was administered at a dose of 5 mg/kg. Such EEG patterns continued till 60–80 min after administration, and thereafter, the patterns recovered to the normal condition (Fig. 5).

b) Effects on arousal responses induced by stimulation to the posterior hypothalamus: When the posterior hypothalamus was stimulated with 200 Hz, 1 msec, 1–4 V, for 5–7 sec, the desynchronization with low-voltage and fast waves appeared at the cortical EEG.

Brotizolam was intraperitoneally injected at doses of 2–5 mg/kg. At a dose of 5 mg/kg, 2 out of 3 rabbits showed moderately inhibited arousal response from 10 to 40 min after administration (Fig. 6). Whereas at a dose of 2 mg/kg, no inhibitory action was clearly detected.

c) Effects on arousal responses induced by stimulation to the midbrain reticular formation: When the ascending activating system of the midbrain reticular formation was stimulated with 100 Hz, 0.75–2 V, for 5–7 sec, the cortical EEG showed arousal responses with low-voltage and fast waves during stimulation.

Brotizolam was intraperitoneally injected at dose of 2–5 mg/kg. At 10–70 min after injection, arousal responses were inhibited clearly in 2 out of 3 rabbits in the 2 mg/kg group and in all 3 rabbits in the 5 mg/kg group. All rabbits became normal at 90–120 min after injection (Fig. 7).

d) Effects on recruiting responses induced by stimulation to the thalamic medial nucleus: The thalamic medial nucleus was stimulated with 6–8 Hz, for 5–7 sec, to

Fig. 4. Effects of brotizolam and other benzodiazepines on the staircase test. Ordinate: rate of climbing and rearing frequency to the control. Abscissa: dose (mg/kg, i.p.). A: brotizolam, B: estazolam and C: triazolam. -- - - : No. of climbing, - - - : No. of rearing.
Fig. 5. Effect of brotizolam on spontaneous EEG. A: control, B: 5 min after intraperitoneal injection of brotizolam 5 mg/kg, C: 20 min and D: 90 min. Abbreviations (Figs. 5–8): I: left, r: right, F: frontal, P: parietal and O: occipital cortex. H: hypothalamus, T: thalamus, H: hippocampus, R: midbrain reticular formation and A: amygdala.

investigate the effects of brotizolam on the diffuse thalamic projecting system. The cortical EEG of untreated rabbits showed recruiting responses. In all 5 rabbits injected intraperitoneally with brotizolam at doses of 2–5 mg/kg, no effect on the system was observed, even up to 180 min after injection.

e) Hippocampal after-discharges: When the dorsal hippocampus of rabbits was stimulated with 100 Hz, 1–5 V, for 5–7 sec, typical hippocampal after-discharges with spikes and waves appeared in the hippocampus, and the after-discharges extended to other areas.
At 10–50 min after intraperitoneal injection of brotizolam, the durations of the after-discharges were shortened in 2 out of 3 rabbits at a dose of 2 mg/kg, while the after-discharges disappeared or were inhibited markedly in all 3 rabbits at a dose of 5 mg/kg (Fig. 8).

9. Tolerance test
On the basis of the spontaneous locomotor activity and pentetrazol-induced convulsions, the tolerance producing liability of brotizolam was tested in mice. Brotizolam at 1.35 mg/kg, p.o., which was five times the ED50 of brotizolam for inhibition of pentetrazol-induced convulsions, was given consecutively to mice and the effects of the drug on these items were examined on the 1st, 7th and 14th day of administration.

a) Spontaneous locomotor activity: As shown in Fig. 9, the effect of brotizolam on the spontaneous locomotor activity recorded by the Animex activity meter was found to be almost the same on days 1, 7 and 14. Thus, no development of tolerance producing liability to the spontaneous locomotor activity was observed. Neither was tolerance developed when estazolam was administered repeatedly at a dose of 3.3 mg/kg.

b) Pentetrazol-induced convulsions: On the 1st, 7th and 14th day of brotizolam administration, 95 mg/kg pentetrazol was subcutaneously injected into the mice, and the appearance of convulsions was investigated. As shown in Table 8, brotizolam continued to show the anticonvulsive effect up to the day 14, without producing tolerance. Also, when estazolam was repeatedly administered at a dose of 3.3 mg/kg, which was five times the ED50 of estazolam for inhibition of pentetrazol-induced convulsions, no tolerance was observed to develop.

Discussion
Brotizolam, a new thieno-triazolo-diazepine derivative, has a chemical structure different from those of benzodiazepine
compounds, but its pharmacological properties are similar to those of triazolam, a benzodiazepine derivative with a triazol ring. In 1979, Koch (17) reported that the drug has a variety of pharmacological actions on the central nervous system such as sedative, anti-anxiety and anticonvulsive effects; production of anti-aggressive behavior; and analgesic, anti-amphetamine and anti-apomorphine actions. For this

![Figure 7](image_url)

**Fig. 7.** Effect of brotizolam on arousal responses induced by stimulation of the midbrain reticular formation. A: control (scale line, 1.25 V), B: 10 min after intraperitoneal injection of 5 mg/kg brotizolam, C: 30 min and D: 90 min.

| Days | Drug   | Dose (mg/kg, p.o.) | No. of animals used | No. of convulsion | total (%) |
|------|--------|--------------------|---------------------|------------------|----------|
|      | Control| 10                 | 10                  | 10 5 10 10       | 10 (100) |
|      | Brotizolam | 1.35           | 10                  | 1 0 0 0         | 1 (10)   |
|      | Estazolam | 3.3             | 10                  | 1 0 0 0         | 1 (10)   |
| 7th  | Control| 10                 | 10                  | 10 6 9 9       | 10 (100) |
|      | Brotizolam | 1.35           | 10                  | 1 0 0 0         | 1 (10)   |
|      | Estazolam | 3.3             | 10                  | 1 0 0 0         | 1 (10)   |
| 14th | Control| 10                 | 10                  | 10 4 9 9       | 10 (100) |
|      | Brotizolam | 1.35           | 10                  | 0 0 0 0         | 0        |
|      | Estazolam | 3.3             | 10                  | 1 0 0 0         | 1 (10)   |
reason, it is generally considered that brotizolam has a neuroleptic action as well as actions specific to benzodiazepine compounds.

In the present study, we obtained results similar to those obtained already in a series of benzodiazepine compounds (18-21) in regard to sedation, decrease in spontaneous locomotor activity, inhibitory effect in the rotarod test, potentiation of pentobarbital-induced sleep, marked inhibition of pentytrazol- and bemebride-induced convulsions and findings from the staircase test. The action of brotizolam in inducing catalepsy...
was also similar to those of prazepam, triazolam and diazepam observed already by Kimishima et al. (19, 20) and Matsuda (13), and brotizolam did not show any neuroleptic action in our study.

Regarding EEG findings, brotizolam tended to cause a change to slow waves in the spontaneous EEG and inhibited arousal responses induced by stimulation to the posterior hypothalamus and midbrain reticular formation, but all of these actions were not different from those of benzodiazepine compounds (18, 19, 21).

As to the potency of this drug, brotizolam was proved to be clearly more potent than diazepam in a series of our experiments. In view of the anticonvulsive action on pentetrazol- and bemegride-induced convulsions, brotizolam was more potent than estazolam, but less potent than triazolam. We could also prove that brotizolam was more potent than estazolam in prolongation of sleep-duration induced by pentobarbital.

In the reports of clinical application of brotizolam, it has been used only as a sleep inducer (12, 22–25). We think that it is appropriate to use brotizolam as a sleep inducer in clinical therapy, considering its strong hypnogenic properties (26), its possession of fewer characteristics of anxiolytic drugs, and speedy metabolism (27, 28).

Toxicity of brotizolam is known to be extremely low. The drug caused acute toxicity in oral administration to mice or rats at doses of 3,000 mg/kg or more (29). In our study on the actions of the drug on the spontaneous locomotor activity and drug-induced convulsions, it exerted these actions without development of tolerance, even if it was administered to animals at a dose five times that of the ED50 for anticonvulsive effects, once daily for 2 consecutive weeks.

For these reasons, brotizolam, a new thieno-triazolo-diazepine compound, is judged to be a safer and stronger sleep inducer than diazepam and estazolam.

References
1. Randall, L.O., Schallek, W., Heise, G.A., Keith, E.F. and Bagdon, R.E.: The psychosedative properties of methamino-diazepoxide. J. Pharmacol. 129, 163–171 (1960)
2 Schallek, W., Zabransky, F. and Kuenn, A.: Effect of benzodiazepines on central nervous system of cat. Arch. Int. Pharmacodyn. Ther. 149, 467–483 (1964)

3 Randall, L.O., Schallek, W., Scheckel, C., Bagdon, R.E. and Rieder, J.: Zur Pharmakologie von Mogadon einem Schlafmittel mit neuartigem Wirkungsmechanismus. Schweiz. Med. Wochenschr. 95, 334–337 (1965)

4 Zbinden, G. and Randall, L.O.: Pharmacology of benzodiazepines; laboratory and clinical correlations. In Adv. Pharmacol, Edited by Garattini, S. and Shore, P.A., Vol. 5, p. 213–291, Academic Press, New York (1967)

5 Randall, L.O., Schallek, W., Scheckel, C., Banziger, R.F. and Moe, R.A.: Pharmakologie des neuen Psychopharmakons 7-chlor-2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepine (Ro 5–4556). Arzneimittelforsch. 18, 1542–1545 (1968)

6 Nakajima, R., Take, Y., Moriya, R., Saji, Y., Yui, T. and Nagawa, Y.: Pharmacological studies on new potent central depressants, 8-chloro-6-phenyl-4H-s-triazolo [4,3-a] [1–4] benzodiazepine (D-40 TA) and its 1-methyl analogue (D-65 MT). Japan. J. Pharmacol. 21, 497–519 (1971)

7 Rudzik, A.D., Hester, J.B., Tang, A.H., Straw, R.N. and Friis, W.: The Benzodiazepines. p. 285, Raven Press, New York (1973)

8 Nakanishi, M., Tsumagari, T., Takigawa, Y., Shuto, S., Kenjo, T. and Fukuda, T.: Studies on psychotropic drugs. XIX: Psychopharmacological studies on new potent central depressants. 8-chloro-6-phenyl-4H-s-triazolo [4,3-a] [1–4] benzodiazepine (D-40 TA) and its 1-methyl analogue (D-65 MT). Japan. J. Pharmacol. 21, 497–519 (1971)

9 Rudzik, A.D., Hester, J.B., Tang, A.H., Straw, R.N. and Friis, W.: The Benzodiazepines. p. 285, Raven Press, New York (1973)

10 Nakajima, R., Take, Y., Moriya, R., Saji, Y., Yui, T. and Nagawa, Y.: Pharmacological studies on new potent central depressants, 8-chloro-6-phenyl-4H-s-triazolo [4,3-a] [1–4] benzodiazepine (D-40 TA) and its 1-methyl analogue (D-65 MT). Japan. J. Pharmacol. 21, 497–519 (1971)

11 Nakajima, R., Take, Y., Moriya, R., Saji, Y., Yui, T. and Nagawa, Y.: Pharmacological studies on new potent central depressants, 8-chloro-6-phenyl-4H-s-triazolo [4,3-a] [1–4] benzodiazepine (D-40 TA) and its 1-methyl analogue (D-65 MT). Japan. J. Pharmacol. 21, 497–519 (1971)

12 Nakajima, R., Take, Y., Moriya, R., Saji, Y., Yui, T. and Nagawa, Y.: Pharmacological studies on new potent central depressants, 8-chloro-6-phenyl-4H-s-triazolo [4,3-a] [1–4] benzodiazepine (D-40 TA) and its 1-methyl analogue (D-65 MT). Japan. J. Pharmacol. 21, 497–519 (1971)

13 Matsuda, A.: Catalepsy induced by the minor tranquilizers, benzodiazepines. The Journal of the Yonago Medical Association 31, 288–301 (1980) (Abs. in English)

14 Woodbury, L.A. and Davenport, V.D.: Design and use of a new electroshock seizure apparatus, and analysis of factors altering seizure threshold and pattern. Arch. Int. Pharmacodyn. Ther. 92, 97–107 (1952)

15 Thiébot, M.H., Soubrié, P., Simon, P. and Boissier, J.R.: Dissociation de deux composantes du comportement chez le rat sous l’effet de psychotropes, application à l’étude des anxiolytiques. Psychopharmacologia (Berlin) 31, 77–90 (1973)

16 Litchfield, J.T. and Wilcoxon, F.: Simplified method of evaluation dose-effect experiment. J. Pharmacol. 96, 99–113 (1949)

17 Koch, H.: Brotizolam, hypnotic agent. Drugs of the Future 4, 85–88 (1979)

18 Kimishima, K., Yamasaki, M., Tanabe, K. and Ogura, C.: Central nervous actions of bromazepam, a new benzodiazepine derivative. The Journal of the Yonago Medical Association 23, 107–116 (1972) (Abs. in English)

19 Kimishima, K., Tanabe, K., Kinoshita, Y., Tamaki, H., Inoue, G., Yogi, H. and Ogura, C.: Central nervous actions of prazepam, a new benzodiazepine derivative. The Journal of the Yonago Medical Association 26, 201–209 (1975) (Abs. in English)

20 Kimishima, K., Tanabe, K., Kinoshita, Y., Tamaki, H., Kawamura, M. and Kurihara, A.: Central nervous actions of triazolam, a new benzodiazepine derivative. The Journal of the Yonago Medical Association 27, 314–323 (1976) (Abs. in English)

21 Kimishima, K., Tanabe, K., Kinoshita, Y., Houri, D., Matsuda, A. and Shishido, H.: Central nervous actions of tofizopam, a new benzodiazepine derivative. The Journal of the Yonago Medical Association 30, 137–147 (1979) (Abs. in English)

22 Grünberger, J., Saletu, B., Linzmayer, L., Kalk, A. and Berner, P.: Pharmacodynamic investigations with WE 941, a new triazolodiazepine, by means of psychometric analysis. Curr. Ther. Res. 24, 427–440 (1978)

23 Kubicki, S.: Elektroenzephalographische Dosisschafprofilbeurteilung eines Hypnotikums aus der Reihe der Triazolothienodiazepine. Z. EEG-EMG 10, 95–100 (1979)

24 Saletu, B., Grünberger, J., Volavka, J. and
Berner, P.: Classification and bioavailability studies with We 941 by quantitative pharmaco-EEG and clinical analysis. Arzneimittelforsch. 29, 700-704 (1979)

25 Fink, M. and Irwin, P.: Pharmacoelectroencephalographic study of brotizolam, a novel hypnotic. Clin. Pharmacol. Ther. 30, 336-342 (1981)

26 Kuhn, F.J., Böke-Kuhn, K., Danneberg, P., Lehr, E. and Stockhaus, K.: Pharmacology and hypnogenic properties of brotizolam in animals. Br. J. Clin. Pharmacol. 16, 253S-260S (1983)

27 Bechtel, W.D.: Pharmacokinetics and metabolism of brotizolam in animals. Br. J. Clin. Pharmacol. 16, 261S-266S (1983)

28 Bechtel, W.D.: Pharmacokinetics and metabolism of brotizolam in humans. Br. J. Clin. Pharmacol. 16, 279S-283S (1983)

29 Hewett, C., Kreuzer, H., Köllmer, H., Niggeschulze, A. and Stötzer, H.: The toxicology of brotizolam. Br. J. Clin. Pharmacol. 16, 267S-274S (1983)