A 7-phenyl substituted triazolopyridazine has inverse agonist activity at the benzodiazepine receptor site

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1 To investigate further the structural requirements for benzodiazepine (BZD) receptor ligands, we synthesized SR 95195, [7-phenyl-3-methyl-1,2,4-triazolo-(4,3-b) pyridazine], a positional isomer of the 6-phenyl-triazolo-pyridazines, which were the first non-BZD derivatives to exhibit high affinity for the BZD receptor and BZD-like activity *in vivo*.

2 *In vitro*, SR 95195 displaced specifically bound [³H]-flunitrazepam from rat cerebellar and hippocampal membranes with respective IC₅₀ values of 4 and 8 μM.

3 *In vivo*, SR 95195 lacked BZD-like activity. At high doses SR 95195 induced clonic seizures in mice (threshold convulsant dose: 150 mg kg⁻¹; CD₅₀: 160 mg kg⁻¹ i.p.) which were antagonized by Ro 15–1788. At non-convulsant doses (25 mg kg⁻¹ i.p. and 100 mg kg⁻¹ i.p.). SR 95195 significantly decreased punished responding in an operant conflict procedure in the rat, suggesting SR 95195 has intrinsic anxiogenic activity.

4 SR 95195, in mice, reversed the anticonvulsant and myorelaxant actions of diazepam 3 mg kg⁻¹, orally (respective ED₅₀ values: 45 mg kg⁻¹ i.p. and 44 mg kg⁻¹ i.p.). In an operant-conflict test in rats, SR 95195 at non-anxiogenic doses, antagonized the disinhibitory action of diazepam 4 mg kg⁻¹, i.p. (ED₅₀: 8.6 mg kg⁻¹, i.p.), but not that of pentobarbitone 15 mg kg⁻¹, i.p.

5 It is concluded that SR 95195 has the pharmacological profile of an inverse BZD agonist and that displacing the phenyl from the 6- to the 7-position in the triazolopyridazine series causes a shift from agonist to inverse agonist type activity at the BZD receptor site.

Introduction

Brain specific benzodiazepine (BZD) binding sites which selectively recognize pharmacologically and clinically active BZDs have been isolated in all vertebrate species including man (Squires & Braestrup, 1977; Möhler & Okada, 1977; Young & Kuhar, 1979; Speth *et al.*, 1978). These receptors also selectively recognize compounds such as Ro 15–1788 (Hunkeler *et al.*, 1981) which reverse all the actions of BZDs or compounds such as CGS 8216 (Boast *et al.*, 1983; File, 1983) which not only antagonize the actions of BZDs, but which exhibit opposite activities.

Early evidence indicated that only a single homogeneous class of BZD binding sites existed. However the discovery of a series of triazolopyridazines, substituted in the 6-position by a

phenyl ring, lent strong support to the concept of multiple BZD receptors. CL 218,872, a member of this series, was the first non-BZD reported to displace [³H]-BZDs from their brain-specific binding sites (Lippa *et al.*, 1979). This compound was found to possess anticonflict and anticonvulsant activities in animals and to produce sedation and myorelaxant effects at doses much higher than the anxiolytic doses (Lippa *et al.*, 1979). Biochemical studies indicated that CL 218,872 preferentially displaced [³H]-BZD from a particular BZD receptor class designated as type I BZD receptor (Klepner *et al.*, 1979). Thus it was suggested that type I receptors mediated the anxiolytic and anticonvulsant actions of BZDs and type II receptors the sedative and myorelaxant actions of BZDs. Recently, however CL 218,872 has been shown to possess sedative properties at anxiolytic doses (Oakley *et al.*, 1984; McElroy *et al.*, 1985) and the

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Figure 1 Chemical structures of SR 95195 and CL218,872.

initial hypothesis according to which different BZD receptor subtypes could mediate different aspects of BZD activities has been questioned (Oakley *et al.*, 1984). Furthermore CL 218,872 has also been shown to reverse diazepam-induced loss of righting reflex (Gee *et al.*, 1983), suggesting together with other results (Gardner, 1984) that this compound may have mixed agonist/antagonist type of activity at the BZD receptor site.

To investigate further the structural prerequisites at the BZD receptor site, we synthesized SR 95195 (7-phenyl-3-methyl-1,2,4-triazolo-(4,3-b) pyridazine; Figure 1; Bourguignon *et al.*, 1985). In this compound, the phenyl ring is attached to the 7-position of the pyridazine ring and not to the 6-position, as is the case for CL 218,872.

The purpose of this study was to examine the influence of this positional isomery in the 6,7-triazolopyridazine series on BZD receptor affinity and on BZD-like action. Diazepam, Ro 15-1788 and CGS 8216 were used as reference benzodiazepine receptor ligands.

Part of this work was presented at the 11th Annual Meeting of the Society for Neuroscience (Chambon *et al.*, 1985).

Methods

Swiss albino CD₁ female mice weighing 18–23 g and male Sprague Dawley rats (200–300 g) were obtained from Charles River Breeding Laboratories (St. Aubin les Elbeuf, France). All animals were weighed, identified one day before the test and housed in groups of 10 (mice) or 5 (rats) in macrolon cages with free access to food and drinking water. A 12 h day/night cycle was maintained in the animal colonies. On the day of the experiment, animals were brought into the laboratory where the test was performed.

For the approach-avoidance conflict experiment rats were housed individually in suspended cages. One

week after arrival, they were progressively food deprived until they received a daily diet of 10–12 g of standard laboratory feed (AO4 UAR) except during week-ends when they were fed *ad libitum* on Saturdays, the food being removed on Sunday mornings. Tap-water was always available as drinking fluid. The room temperature was $21^{\circ}\text{C} \pm 1$, with a relative humidity of $50 \pm 10\%$. The light-dark cycle was 12 L/12 D, lights being on at 07 h 00mm.

In vitro [^3H]-flunitrazepam binding

In vitro [^3H]-flunitrazepam binding was performed according to a slight modification of the method described by Stapleton *et al.* (1982). Rats were stunned and decapitated, their brains were rapidly removed, the cerebella and hippocampi were removed and homogenized in 50 mM Tris-citrate buffer (pH 7.1) with a Brinkman Polytron PT 10 homogenizer. Homogenates were centrifuged at 48,000 g for 10 min at 4°C . The pellets were again homogenized, centrifuged twice and frozen at -80°C for at least 24 h. On the day of the experiment the membranes were thawed, homogenized in 100 vol of 25 mM KH_2PO_4 pH 7.1, centrifuged (48,000 g, 4°C , 10 min) and resuspended twice in the same buffer. Aliquots of membrane suspensions (0.25 mg protein) were incubated in 1 ml of 25 mM KH_2PO_4 (pH 7.1) with 1.05 nM [^3H]-flunitrazepam (80 Ci mmol^{-1}) and various concentrations of potential radioligand displacers for 90 min at 4°C . The assay was terminated by filtration under vacuum on Whatman GF/C filters. Filters were washed with 10 ml of ice-cold buffer, placed in scintillation vials and allowed to dry overnight; 10 ml of scintillation fluid (Biofluor; New England Nuclear, Boston, USA) was then added and radioactivity was counted in a Kontron Betamatic liquid scintillation counter. Specific binding of [^3H]-flunitrazepam was defined as the difference between total binding in the presence of radioligand alone and non-specific binding in the presence of 100 μM unlabelled diazepam.

Gross behaviour and convulsant effects in mice

SR 95195, or vehicle, was administered i.p. to groups of 10 mice, or orally to groups of 5 rats. Animals were observed grouped in their treatment cage. The symptomatic scale described by Irwin (1968) was used. This scale provides information on central symptoms such as motor activity, tremor, convulsions, stereotypies and peripheral automatic symptoms such as pupillary diameter, ptosis, salivation, body temperature, respiration. The symptoms were noted as present or absent during the 3 h after drug administration; no scoring system was used and the observer was not blind to treatment conditions. Lethality was recorded up to 72 h after drug administration. The CD_{50} (95% confidence limits), dose which induced clonic seizures in 50% of animals, was calculated by probit analysis (Litchfield & Wilcoxon, 1949).

Antagonism of SR 95195-induced seizures by Ro 15-1788

Groups of 10 mice were used. Ro 15-1788 was injected i.p. at doses ranging from 1.25 mg kg^{-1} to 20 mg kg^{-1} ; 5 min later SR 95195 was injected at a dose of 180 mg kg^{-1} , a dose which induced clonic seizures in 90% of animals. The number of mice exhibiting clonic seizures was recorded 1 h after the administration of SR 95195.

Reversal of the anticonvulsant effects of diazepam in mice

Experiments were carried out in groups of 10 mice. Diazepam was administered orally at 3 mg kg^{-1} 30 min before an i.p. injection of test compounds or vehicle and 60 min before pentylenetetrazole (Ptz) 100 mg kg^{-1} , s.c. The number of animals exhibiting seizures in each treatment group was recorded 1 h after the administration of Ptz. All animals pretreated by diazepam alone were protected against seizures. The ED_{50} (95% confidence limits), dose which antagonized the anticonvulsant effect of diazepam in 50% of animals, was calculated for each test compound by probit analysis (Litchfield & Wilcoxon, 1949).

Reversal of the myorelaxant effects of diazepam in mice

Myorelaxation was measured in the Traction test according to a procedure similar to that described by Boissier *et al.* (1961). The test was performed using a metallic wire (diameter 1 mm; length 15 cm) fixed horizontally 20 cm above the experimental table. Groups of 10 mice were used. Diazepam (3 mg kg^{-1}) was injected i.p. 30 min before the i.p. injection of test compounds or vehicle. One hour later animals were

suspended by their fore-paws from the metallic wire and the number of mice failing to put one hind-limb on the wire during the following 5 s was counted. All animals pretreated by diazepam alone failed to perform the test. The ED_{50} (95% confidence limits), dose which antagonized the myorelaxant effect of diazepam in 50% of animals, was calculated for each test compound by probit analysis (Litchfield & Wilcoxon, 1949).

Approach-avoidance conflict test in rats

Conditioning was carried out in Skinner boxes (Campden, London, U.K.; ref. 4104) isolated by sound-attenuating chambers. Each Skinner box was supplied with a food tray connected with a pellet dispenser, with one lever above which an electric bulb was located and with a grid floor which was connected to a shock generator and scrambler (Campden, London, U.K. ref. 521C). Operant schedule and animal responding were monitored by a microcomputer (Apple II, Apple Computer Inc., Cupertino, U.S.A.).

Experiments were run from 13 h 00 min to 17 h 00 min. Two weeks after arrival, rats were familiarized with the experimental chambers and trained to press the lever in order to obtain one 45 mg food pellet (Bioserv Inc., Frenchtown, U.S.A.). When they steadily pressed the lever, they were submitted to a progressively increasing variable interval (VI) schedule, until the final VI 30 s schedule was reached (i.e. a food reinforcement was available at the end of a period of time randomly varying from 5 s to 55 s). The training sessions lasted 30 min. When baseline responding was stable, two fixed ratio 3 (FR 3) segments were included, they were cued by the onset of the lamp above the lever. During the FR3 segments, every third press delivered simultaneously a food pellet and an electric shock. The shock intensity was titrated to each animal's sensitivity so that no more than 6 punished lever presses were made per session. Sessions were organized according to the following sequence: 10 min VI 30 s, 2 min FR3, 10 min VI 30 s, 2 min FR3 and 6 min VI 30 s. Animals were trained 4 days a week. The possible anticonflict effect of drugs was examined when the rats were well trained in the procedure and when steady performances were achieved. They were carried out on two different days a week and were separated by at least one training day.

When studied alone, drugs (i.e. SR 95195 and CL218,872) were injected i.p. 30 min before testing. When administered in combination with diazepam or pentobarbitone, SR 95195 was injected i.p. 60 min before the beginning of the session and diazepam (4 mg kg^{-1}) or pentobarbitone (15 mg kg^{-1}) were injected i.p. 30 min before testing. Control rats were dosed with the corresponding vehicle of each drug. The order of treatment was randomized for each rat.

The total number of responses during the non-punished and the punished periods of the conflict test were recorded. Punished responses (PR) were submitted to a logarithmic transformation [$\log(\text{PR} + 1)$] and non-punished responses (NPR) were expressed as the ratio of response rate on the test day to the response rate on the day preceding drug treatment (NPR/NPR_0). Results were submitted to an ANOVA for repeated measures if variances were assumed to be equal (Levene's test) or to Brown-Forsythe's test otherwise. Comparisons between the means were done by Bonferroni's method. Whenever possible ED_{50} values were calculated by the method of Finney (1971). Rats were considered to be disinhibited when they made more than seven PR per session.

Drugs

Flunitrazepam ([methyl- ^3H]; 80 Ci mmol $^{-1}$) was purchased from N.E.N. (Boston, U.S.A.). SR 95195 was synthesized at the Laboratoire de Pharmacochimie Moléculaire, UA 501, Centre de Neurochimie du CNRS, U.L.P. (Strasbourg, France). CL 218,872 was synthesized at the Department of Organic Chemistry of the Centre de Recherches Clin-Midy, Sanofi Recherche (Montpellier, France). Ro 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imadazol (1, 5a)-(1,4) benzodiazepine-3-carboxylate) and diazepam were provided by Hoffman-la-Roche (Basel, Switzerland). CGS 8216 (2-phenylpyrazolo (4,3-c) quinolin-3 (5H)-one) was provided by Ciba-Geigy (Summit, U.S.A.), pentobarbitone was purchased from Interchim (Montluçon, France) and pentylene-tetrazole from Sigma (Saint Louis, U.S.A.).

Mice were dosed orally with diazepam suspended in 5% gum tragacanth (w/v), i.p. with diazepam suspended in 0.5% carboxymethyl cellulose (w/v) or s.c. with pentylene-tetrazole dissolved in distilled water. All other drugs were suspended in 0.5% carboxymethyl cellulose (w/v) and injected i.p. In the approach-avoidance conflict test in rats, all the drugs tested were suspended in 0.5% carboxymethyl cellulose (w/v) with two drops of Tween 80 per 10 ml and injected i.p.

Results

In vitro [^3H]-flunitrazepam binding

SR 95195 displaced bound [^3H]-flunitrazepam from rat cerebellar and hippocampal membranes with IC_{50} values in the micromolar range (Table 1). SR 95195 appeared to be 10 times and 60 times less potent than CL 218,872 in hippocampal and cerebellar membranes respectively. It was considerably less potent than diazepam, Ro 15-1788 and CGS 8216 in both regions (Table 1). Unlike CL 218,872 but like diazepam,

Table 1 Inhibition of *in vitro* [^3H]-flunitrazepam binding in rat cerebellum and hippocampus by SR 95195 and various benzodiazepine receptor ligands

Compounds	IC_{50} (nM)	
	Cerebellum	Hippocampus
SR 95195	4860 $\pm$ 580	8500 $\pm$ 1300
CL 218,872	76 $\pm$ 7	866 $\pm$ 30
Diazepam	3.3 $\pm$ 1.8	3.2 $\pm$ 1.9
Ro 15-1788	2.2 $\pm$ 1.0	2.3 $\pm$ 1.0
CGS 8216	0.0063 $\pm$ 0.0013	0.0039 $\pm$ 0.0013

Data are expressed as the mean $\pm$ s.e.mean of 3 individual experiments performed in triplicate on separate days.

Ro 15-1788 and CGS 8216, SR 95195 exhibited comparable affinities for the cerebellar and hippocampal BZD receptor site (Table 1). Hill plots of the saturation isotherms of [^3H]-flunitrazepam binding in cerebellar and hippocampal membranes revealed Hill numbers of 0.85 ± 0.03 and 0.73 ± 0.03 respectively.

Gross behaviour and convulsant effect in mice

When administered i.p. to mice, SR 95195 did not induce BZD-like activity; in particular no intoxicated gait and no sedation were observed. At high doses, SR 95195 induced clonic seizures ($\text{CD}_{50} = 160 \text{ mg kg}^{-1}$ i.p., 95% confidence limits: 148–175; threshold convulsant dose: 150 mg kg^{-1} i.p.) followed by death ($\text{LD}_{50} = 240 \text{ mg kg}^{-1}$ i.p., 95% confidence limits: 220–261).

Antagonism of SR 95195-induced seizures by Ro 15-1788

SR 95195 at a dose of 180 mg kg^{-1} induced clonic seizures in 90% of mice, which were antagonized by

Table 2 Reversal by SR 95195 and reference compounds of the anticonvulsant and muscle relaxant effects of diazepam (3 mg kg^{-1} orally)

Compounds	ED_{50} (mg kg^{-1} , i.p.)	
	Ptz-induced seizures	Traction test
SR 95195	45 (30–58)	44 (29–67)
Ro 15-1788	17 (14–21)	3.6 (2.6–4.8)
CGS 8216	0.97 (0.20–4.40)	0.87 (0.70–1.04)

Ptz = pentylene-tetrazole

Figures in parentheses indicate the 95% confidence limits.

Figure 2 Dose-response curves of SR 95195 on punished (PR ○) and non-punished responding (NPR ●) in the approach-avoidance conflict test. SR 95195 was injected i.p. 30 min before testing. Comparisons between the means were made by Bonferroni's method. Significance is indicated by * for PR and * for NPR (* $P < 0.05$; ** $P < 0.01$).

Ro 15-1788 ($ED_{50} = 3.8 \text{ mg kg}^{-1}$ (1.3–10.6)). Comparable results were obtained in two different experiments.

Figure 3 Dose-response curves of CL 218,872 on punished (PR ○) and non-punished responding (NPR ●) in the approach-avoidance conflict test. CL 218,872 was injected i.p. 30 min before testing. Comparisons between the means were made by Bonferroni's method. Significance is indicated by * for PR and * for NPR (* $P < 0.05$; ** $P < 0.01$).

Reversal of the anticonvulsant effects of diazepam in mice

Diazepam at a dose of 3 mg kg^{-1} orally, completely blocked the clonic seizures induced by Ptz (100 mg kg^{-1} s.c.) administered 1 h later. In these conditions, when SR 95195 was administered i.p. at non-convulsant doses (25 to 100 mg kg^{-1}) 30 min after diazepam, it antagonized the protective effect of diazepam against Ptz-induced clonic seizures in a dose-dependent fashion ($ED_{50} = 45 \text{ mg kg}^{-1}$). Ro 15-1788 and CGS 8216 also antagonized the anticonvulsant effect of diazepam and were 2.5 times and 50 times more potent respectively than SR 95195 (Table 2).

Reversal of the myorelaxant effect of diazepam in mice

Diazepam at a dose of 3 mg kg^{-1} i.p. induced muscle relaxation in all animals as measured by the traction test, 60 min after injection. When SR 95195 was administered i.p. at various doses, 30 min after

Figure 4 Dose-response curves of SR 95195 on punished (PR ○) and non-punished responding (NPR ●) after injection of diazepam (4 mg kg^{-1} , i.p.) in the approach-avoidance conflict test. SR 95195 was injected i.p. 60 min before testing and 30 min before diazepam. Each point represents the mean with vertical lines showing s.e.mean. Comparisons between the means were made by Bonferroni's method. Significance is indicated by * for PR, they are located above the graph for comparisons with controls and below the graph for comparison with the agonist (* $P < 0.05$; *** $P < 0.001$).

diazepam, it dose-dependently antagonized the muscle relaxation induced by diazepam ($ED_{50} = 44 \text{ mg kg}^{-1}$). In the same experimental conditions, Ro 15-1788 and CGS 8216 also antagonized the myorelaxant effects of diazepam and were 12 times and 50 times more potent respectively than SR95195 (Table 2).

Approach-avoidance conflict test in rats

The activity of SR95195 in the approach-avoidance conflict test is presented in Figure 2. SR95195 significantly modified PR (ANOVA: $F(4-25) = 3.6$, $P < 0.05$). Comparisons between the means indicated that PR was reduced at 25 mg kg^{-1} and 100 mg kg^{-1} . SR95195 also decreased NPR (ANOVA: $F(4-25) = 15.4$, $P < 0.001$); this effect was significant only at 100 mg kg^{-1} . In contrast and as expected, CL218,872 (Figure 3) increased PR (ANOVA: $F(4-25) = 5.65$, $P < 0.01$). Comparisons between the means showed that the means of all doses were significantly superior to control values. The ED_{50} value for the disinhibitory action of CL218,872 was 25.3 mg kg^{-1} . CL218,872 also significantly decreased NPR (Brown-Forsythe's test = 28.0 for 4-17 d.f., $P < 0.001$) at 20 and 40 mg kg^{-1} .

As shown in Figure 4, diazepam (4 mg kg^{-1}) significantly increased PR and this effect was dose-dependently antagonized by SR95195 (Brown-Forsythe's test = 15.5 for 4-24 d.f., $P < 0.001$; $ED_{50} = 8.6 \text{ mg kg}^{-1}$). NPR was not modified by the treatment (Figure 4; ANOVA: $F(4-30) = 0.84$; $P > 0.05$).

Pentobarbitone (15 mg kg^{-1}) increased PR (ANOVA: $F(3-24) = 5.47$; $P < 0.01$), this effect was not antagonized by SR95195 (6.25 and 25 mg kg^{-1}) and no group injected with SR95195 differed significantly from the pentobarbitone-treated group (Figure 5). No change in NPR was recorded (ANOVA: $F(3-24) = 0.28$; $P > 0.05$).

Discussion

The results presented in this study clearly showed that SR95195 has the pharmacological profile of an inverse BZD agonist. *In vitro*, SR95195 displaced specifically bound [^3H]-flunitrazepam from its receptor site. *In vivo*, SR95195 lacked BZD-like activity, but was able to reverse the anticonvulsant, myorelaxant and anxiolytic actions of diazepam. Moreover SR95195 appeared to have intrinsic anxiogenic actions and at high doses induced clonic seizures.

The affinity of SR95195 for the BZD receptor site was considerably lower than that observed with the other reference compounds examined in this study. Moreover, unlike CL218,872, but like the other reference compounds, SR95195 displaced [^3H]-flunitrazepam from cerebellar and hippocampal mem-

Figure 5 Dose-response curves of SR 95195 on punished (PR ○) and non-punished responding (NPR ●) after injection of pentobarbitone (15 mg kg^{-1} , i.p.) in the approach-avoidance conflict test. SR 95195 was injected i.p. 60 min before testing and 30 min before pentobarbitone. Each point represents mean with vertical lines showing s.e.mean. Comparisons between the means were made by Bonferroni's method. Significance is indicated by * for PR (* $P < 0.05$; *** $P < 0.001$).

branes with comparable potencies. The chemical structure of SR95195 differs from that of CL218,872 not only in the position of the phenyl ring, but also in the absence of a CF_3 substituent on the phenyl. However, in the 6-phenyl-triazolopyridazine series the compound which is not substituted on the phenyl ring has been shown to have the same pharmacological profile as CL218,872, but to be slightly less potent (Albright *et al.*, 1981). Thus it can be concluded that displacing the phenyl from the 6- to the 7-position in the triazolopyridazine series causes a marked decrease in affinity for the BZD receptor. Moreover, this structural modification also leads to a loss of selectivity for type I BZD receptor sites, as indicated by comparable affinities for the cerebellar and hippocampal BZD receptors.

SR95195, at doses higher than 150 mg kg^{-1} , induced clonic seizures which were blocked by Ro 15-1788, a BZD receptor antagonist, indicating that seizure activity was probably mediated by the BZD receptor. Inverse BZD agonists have been shown either to induce seizures or to have definite proconvulsant actions (Cowen *et al.*, 1981; Oakley & Jones, 1982; Braestrup *et al.*, 1982; File, 1983) whereas the BZD receptor antagonist, Ro 15-1788 does not lower seizure threshold (Nutt *et al.*, 1982). Thus the induc-

tion of seizures by SR95195 strongly suggests this compound is an inverse BZD agonist.

Consistent with this hypothesis, in the approach-avoidance conflict test in rats, SR95195 induced a significant decrease in PR at 25 mg kg⁻¹ and 100 mg kg⁻¹ suggesting a possible proconflict action. An enhancement of shock-induced suppression has already been described for inverse agonists such as CGS8216 (Petersen *et al.*, 1983; File & Lister, 1983), β -CCM (De Carvalho *et al.*, 1983) and the endogenous diazepam binding inhibitor (Guidotti *et al.*, 1983). Ro 15-1788, a BZD antagonist, originally considered as devoid of intrinsic pharmacological activity, has also been shown to be anxiogenic when administered alone, however, this effect is weak and disappears at high doses (File *et al.*, 1982).

SR95195, at non-convulsant doses, reversed the anticonvulsant and myorelaxant effects of diazepam in mice. Similar findings have been reported for other BZD inverse agonists and for the BZD antagonist, Ro 15-1788 (Tenen & Hirsch, 1980; Cowen *et al.*, 1981; Nutt *et al.*, 1982; Oakley & Jones, 1982; Czernik *et al.*, 1982). SR95195, at low non-anxiogenic doses, also reversed the anticonflict action of diazepam. Again, inverse BZD agonists and BZD antagonists have repeatedly been shown to antagonize the anxiolytic effect of BZD ligands (Bonetti *et al.*, 1982; Patel *et al.*, 1983). SR95195, unlike CGS8216 (Mendelson *et al.*, 1983) failed to counteract the disinhibitory effect

of pentobarbitone in the approach-avoidance conflict test in rats, suggesting a greater selectivity towards the BZD receptor. Interestingly, the possibility that CGS8216 may also interact with the picrotoxin site has already been suggested on the basis of its proconvulsant mechanism of action (File, 1983).

In conclusion the results presented in this study have shown that displacing the phenyl from the 6- to the 7-position in the triazolopyridazine series causes a shift from an agonist to an inverse agonist type of activity at the BZD receptor site. Minor structural changes have previously been shown in other BZD receptor ligand series to alter the pharmacological profile of a ligand. Thus, CGS8216, an inverse agonist at the BZD receptor site, differs from CGS9896, a full agonist, by an additional chlorine in the 4-position on the phenyl ring (Petrack *et al.*, 1983). In the β -carboline series, addition of a bulky substituent in the 5-position, as is the case for ZK91296, leads to an anticonvulsant compound (Meldrum *et al.*, 1983). However SR95195 is the first example in which positional isomery leads to an inversion of activity.

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