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Preclinical Studies With Pyrazolopyridine Non-Benzodiazepine Anxiolytics: ICI 190,622

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PATEL, J. B., B. A. MEINERS, A. I. SALAMA, J. B. MALICK, D. C. U'PRICHARD, R. E. GILES, M. E. GOLDBERG AND T. M. BARE. *Preclinical studies with pyrazolopyridine non-benzodiazepine anxiolytics: ICI 190,622.* PHARMACOL BIOCHEM BEHAV **29**(4) 775–779, 1988.—Tracazolate is a pyrazolopyridine anxiolytic that enhances the binding of [^{3}H]-flunitrazepam ([^{3}H]FLU) to brain tissue. The discovery that a metabolite of tracazolate, desbutyltracazolate, was a weak inhibitor of [^{3}H]FLU binding led to the synthesis of a series of potent anxiolytics. From this series, ICI 190,622 emerged as a viable drug candidate, being a potent anxiolytic in rats and monkeys. This anxiolytic agent appears to produce only minimal sedation. Furthermore, ICI 190,622 appears less likely to potentiate the actions of ethanol than diazepam. ICI 190,622 is also a potent anticonvulsant (anti-metrazol ED_{50}=1.1 mg/kg, PO) in rodents. Neurochemically, ICI 190,622 is similar to the benzodiazepine anxiolytics. *In vitro*, ICI 190,622 competitively inhibited [^{3}H]FLU binding in cerebral cortex with an IC_{50} of 81 nM and was 4.3-fold more potent in the cerebellum (IC_{50}=19 nM). This suggests a selectivity for the Type 1 benzodiazepine binding site. In contrast, diazepam showed similar affinities in both regions (cerebral cortex=7 nM and cerebellum=9 nM). Following oral administration, ICI 190,622 displaced [^{3}H]FLU binding from cerebellar membranes more potently than diazepam (ED_{50}=3 and 6 mg/kg, respectively, 1 hour after administration). Thus, ICI 190,622 should be an effective anxiolytic with significant advantages over benzodiazepines.

Non-benzodiazepine Anxiolytic Rats Monkey Binding Receptor

ALTHOUGH the benzodiazepines have been the agents of choice in the treatment of anxiety, concerns over the side effects (sedation, alcohol interaction, and dependence liability) have focused new research into non-benzodiazepine agents. To this end, several non-benzodiazepine compounds have been reported as potential anxiolytics; these agents include tracazolate, alpidem [10], zopiclone, CGS 9896, CL 218,872, and buspirone. Of these, alpidem, zopiclone, CGS 9896, and CL 218,872 displace [^{3}H]benzodiazepines, whereas buspirone is a non-displacer. Tracazolate is atypical in that it has been shown to enhance the binding of [^{3}H]FLU to brain tissue [8]. The discovery that desbutyltracazolate, a minor metabolite and thermal degradation product of tracazolate, was a weak inhibitor of benzodiazepine binding led to a series of potent anxiolytics. This report describes the pharmacological profile of one such compound, ICI 190,622, a pyrazolopyridine carboxamide (Fig. 1), a non-benzodiazepine that is anxioselective in that it exhibited antianxiety activity at much lower doses than those that produced sedation.

METHOD

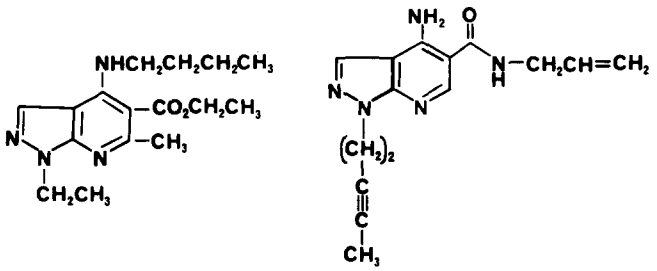
Detailed descriptions of the methods used in these studies are appropriately referenced. The animals used in these experiments were male Wistar (Hilltop Laboratories, Scottdale, PA) rats (180–220 g) and male Swiss-Webster (HLA) mice (18–25 g). All compounds were suspended in an HPMC vehicle (0.1% Tween 80, 0.5% hydroxypropyl methylcellulose in 0.9% NaCl). Drugs were administered in a volume of 5 ml/kg for rats and 20 ml/kg for mice.

Neuropharmacological Effects

Antianxiety activity.

Shock-induced suppression of drinking (SSD) test in rats. The procedure used is a modification [11] of the method developed by Vogel and coworkers [17]. Rats were administered either vehicle or drug orally and tested 60 minutes later. The anticonflict activity of diazepam and ICI 190,622 is presented in Fig. 2. Diazepam exhibited significant ($p<0.05$) increases in the mean number of shocks taken over a wide range of doses (5.0–20.0 mg/kg). Likewise, ICI 190,622 demonstrated a dose-dependent increase in the mean number of shocks taken; significant ($p<0.05$) increases were obtained from 0.8–12.5 mg/kg, PO. Thus, ICI 190,622 was approximately six times as potent as diazepam in this procedure.

Activity in primates: Squirrel monkey conflict test. The antianxiety activity of compounds in a non-human primate was determined using the Squirrel Monkey Conflict Test described by Patel and Migler [12]. Briefly, the test session was



TRACAZOLATE **ICI 190,622**

FIG. 1. Chemical structures of tracazolate and ICI 190,622.

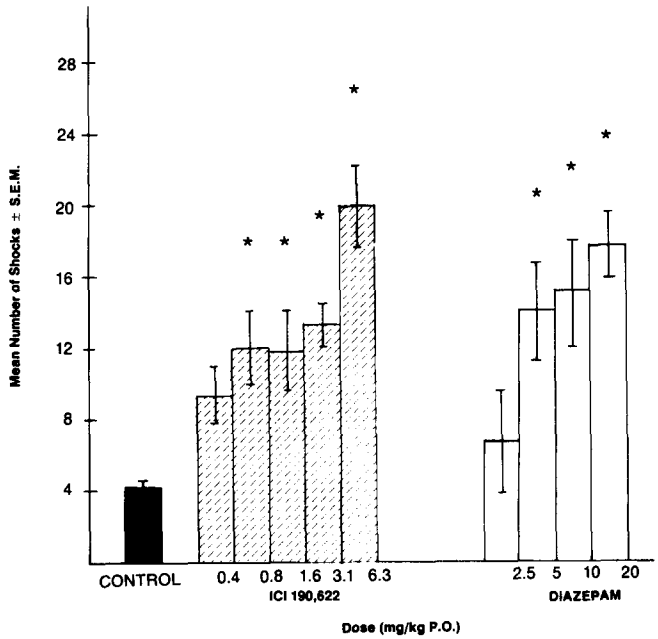


FIG. 2. Comparative effects of diazepam (open column) and ICI 190,622 (diagonally slanted line column) following oral administration in the SSD test in rats. N=8/group. *$p<0.05$; Student's t-test as compared to respective same-day vehicle control.

divided into two portions: (1) a conflict period (first 3 hours) during which each correct response was both reinforced and simultaneously suppressed by a brief mild shock and (2) a reward period (the last 3 hours) during which each correct response was just reinforced by food. The number of shocks taken during the conflict period was recorded and used as a measure of inhibition or "anxiety." The antianxiety effects of diazepam and ICI 190,622 in the monkey conflict test are summarized in Table 1. The number of shocks taken during pre-drug and drug sessions is shown for comparative purposes. In general, the data from this procedure do not usually permit ED_{50} or MED determination since individual monkeys react very differently to given doses of drugs (i.e., their sensitivities vary greatly). Diazepam exhibited significant disinhibitory effects (i.e., subjects took significantly higher numbers of shocks during the conflict period). As shown in Table 1, five out of six monkeys took a significant

TABLE 1

EFFECT OF ICI 190,622 AND DIAZEPAM IN THE SQUIRREL MONKEY CONFLICT TEST

		Number of Shocks Taken During Punished Period			
		ICI 190,622		Diazepam	
Monkey No.	Dose (mg/kg, PO)	Pre-Drug	Drug	Pre-Drug	Drug
29	0.39			0	0
	0.78			0	48
	1.56	0	0	0	141
	3.125	0	13		
	6.25	0	83		
31	0.78			0	1
	1.56	0	0	0	62
	3.125	1	11		
	6.25	0	18		
43	0.78			0	0
	1.56			0	16
	3.125	0	0	0	8
	6.25	0	102	0	74
45	1.56			0	0
	3.125			0	102
	6.25	0	27		
	12.5	0	77		
46	0.2			0	120
	0.39	0	2	0	118
	0.78	1	110	2	191

number of shocks at or below the 1.6 mg/kg dose. Similarly, ICI 190,622 was a very effective anticonflict agent in the monkey (Table 1). Five out of six monkeys took a significant number of shocks in the dose range of 0.78–6.25 mg/kg, PO.

Anticonvulsant activity. Rats weighing 200–260 g were used for these studies. Groups of six rats each were challenged with either metrazol (75.0 mg/kg, SC) or bicuculline (3.0 mg/kg, SC) 45 minutes after an oral administration of vehicle, diazepam, or ICI 190,622. The details of these procedures were described previously [11]. The results of these studies are summarized in Table 2. As expected, diazepam demonstrated activity (ED_{50}=8.4 mg/kg, PO) as an antagonist of metrazole-induced convulsions in rats. Compound ICI 190,622 was extremely potent (ED_{50}=1.1 mg/kg, PO) as an antagonist of metrazole-induced convulsions, being approximately eight times as potent as diazepam.

In the bicuculline-induced convulsion test in rats, diazepam demonstrated significant antagonist activity with an ED_{50} of 9.3 mg/kg, PO (Table 2). ICI 190,622 also exhibited dose-related, extremely potent (ED_{50}=1.4 mg/kg, PO) anticonvulsant activity in this test, being approximately 7 times as potent as diazepam.

Sedative liability. The rotorod test was used to assess comparative effects on sedation and neuromuscular incoordination (ataxia) of diazepam and ICI 190,622 (see Table 3). Diazepam produced ataxia at relatively low doses (ED_{50}=10.2 mg/kg, PO). In contrast, ICI 190,622 only exhibited impairment of rotorod performance at higher doses (ED_{50}=25.1 mg/kg, PO). As shown in Table 3, the sedative

TABLE 2

MEDIAN EFFECTIVE DOSES (ED_{50}) FOR ANTICOVULSANT ACTIVITY IN RATS

Treatment	n*	ED_{50} mg/kg, PO (95% confidence limits) Metrazol (75.0 mg/kg, SC)	Bicuculline (3.0 mg/kg, SC)
Diazepam	30	8.4 (4.8–14.2)	9.3 (5.9–13.6)
ICI 190,622	24	1.0 (0.8–1.2)	1.4 (0.7–2.6)

*Number of rats tested.

TABLE 3

SEDATIVE LIABILITY INDEX FOR DIAZEPAM AND ICI 190,622 IN RATS

Treatment	SSD* (MED; mg/kg, PO)	Rotorod† (ED_{50}; mg/kg, PO)	Sedative Liability Index Rotorod ED_{50} SSD MED
Diazepam	5.0	10.2	2.0
ICI 190,622	0.8	25.1	31.4

*MED for disinhibition in the Shock-Induced Suppression of Drinking Test.

†ED_{50} for impairment of rotorod performance.

TABLE 4

COMPARATIVE EFFECTS OF DIAZEPAM AND ICI 190,622 ON ETHANOL-INDUCED IMPAIRMENT OF ROTOROD PERFORMANCE IN RATS

Treatment*	N†	ED_{50} (mg/kg, PO) for Impairment of Rotorod Performance (95% confidence limits)‡	Ethanol-Interaction Ratio§
Diazepam + Ethanol	24	2.5 (1.4–4.5)	0.5
ICI 190,622 + Ethanol	40	3.1 (2.6–3.8)	3.9

*Rats were treated orally with test agent 15 minutes prior to ethanol (0.8 g/kg, IP).

†Number of rats tested.

‡ED_{50} and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon.

§Ratio = $\frac{\text{Drug + Ethanol (FMA } ED_{50})}{\text{SSD (MED)}}$

TABLE 5

DISPLACEMENT OF [^{3}H]FLU FROM RAT BRAIN MEMBRANES BY ICI 190,622 AND REFERENCE ANXIOLYTICS

Compound	IC_{50} (nM) Cortex	Cerebellum	Ratio (cortex/cerebellum)
ICI 190,622	81	19	4.3
Diazepam	7.3	9.3	0.78
CL 218,872	233	53	4.4

The displacement of specific [^{3}H]FLU (0.2 nM) binding from a washed crude mitochondrial fraction (P2) of the noted brain regions at 0°C was measured as described in the text.

liability index for diazepam was 2.0, whereas it was 31.4 for ICI 190,622. These data indicate that ICI 190,622 is likely to be a much more selective anxiolytic (i.e., anxioselective) than the benzodiazepines in general use in that it exhibits therapeutic effects at much lower doses than those at which sedative properties occurred.

Interaction with ethanol. In addition to possessing anxiolytic efficacy, it is also very beneficial if a novel anxiolytic exhibits less adverse interactions with ethanol as well as reduced sedative liability. Thus, the rat rotorod (ataxia) test was used to determine the ethanol-potentiation liabilities of both diazepam and ICI 190,622. In this study, trained rats were treated orally with either vehicle or test agent 15 minutes prior to the administration of ethanol (0.8 g/kg, IP). All rats were then retested on the rotorod at 15, 30, and 60 minutes after ethanol. The ED_{50} for diazepam to further impair rotorod peformance in ethanol-treated rats was 2.5 mg/kg, PO. ICI 190,622 exhibited an ED_{50} of 3.1 mg/kg, PO for rotorod impairment in combination with ethanol. As shown in Table 4, the ethanol-interaction ratio was 0.5 for diazepam, whereas for ICI 190,622 it was 3.9. These results suggest that ICI 190,622 should be considerably less likely to cause adverse interactions with ethanol compared to diazepam at therapeutic doses in man.

Neurochemical Properties

There is a good correlation between the affinity of the benzodiazepine anxiolytics for their binding site in brain and their clinical potency [1]. Likewise, there is a good correlation between affinity at the benzodiazepine binding site and the activity in animal models such as anticonflict procedures and anticonvulsant tests used to predict anxiolytic activity [4,6]. Displacement of [^{3}H]FLU binding from rat brain synaptic membranes, as described by Meiners and Salama [8], was used to determine the affinity of ICI 190,622 for the benzodiazepine receptor. In the cerebral cortex of rat, ICI 190,622 was found to be a potent displacer of [^{3}H]FLU binding *in vitro*. However, it was less potent than diazepam (IC_{50} values=81 and 7 nM, respectively; Table 5).

The existence of two or more classes of benzodiazepine receptors was originally suggested by Lippa and coworkers and, in recent years, a rather large body of supportive data has been accumulated [3, 5, 14], although there is by no means universal agreement [7]. The different types of receptors are not uniformly distributed in the brain, the cerebellum having almost pure Type 1 receptor whereas the cerebral cortex has a mixture of Type 1 and Type 2 receptors. The benzodiazepines in general use appear to have the same affinity for both classes of receptor, and for this reason, they show Hill coefficients of about unity and equal affinities in different brain regions. ICI 190,622 differs from the benzodiazepine anxiolytics in that it is more potent in the cerebellum than in the cerebral cortex (Table 5). This suggests that ICI 190,622 has a higher affinity for the Type 1 receptor

TABLE 6

GABA SHIFT IN THE DISPLACEMENT OF [^{3}H]FLU BINDING

Compound	IC_{50} (nM) No GABA	IC_{50} (nM) 100 μM GABA	Ratio (no GABA/GABA)
ICI 190,622	192	144	1.3
Diazepam	46	28	1.6
CL 218,872	253	162	1.6
Ro15-1788	6.3	6.3	1.0
BCCE	5.5	5.6	1.0
CGS 8216	0.42	0.58	0.7

The displacement of specific [^{3}H]FLU (1 nM) binding by various agents from a washed, frozen, and thawed crude mitochondrial (P2) fraction of cerebellar tissue was measured in the presence or absence of GABA (100 μM) at 37°C.

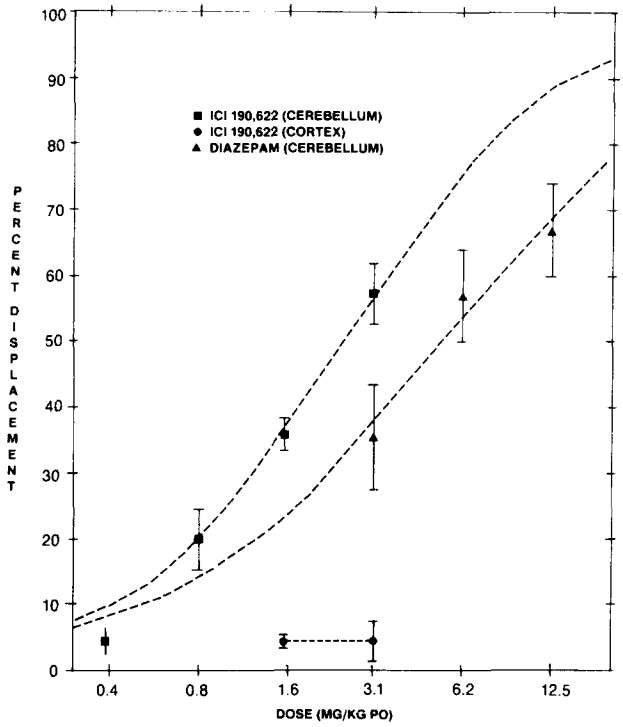


FIG. 4. *Ex vivo* binding in rat brain. Different doses of ICI 190,622 or diazepam were administered orally to rats which were decapitated 60 min later. The brains were dissected and frozen on dry ice. Subsequently, the specific binding of [^{3}H]FLU (1 nM) was measured in crude homogenates (20 mg/ml) of the noted brain regions. The data are expressed as a percentage of the specific binding to the tissue from vehicle-treated rats. The individual points represent the average of the results for 5 to 12 rats.

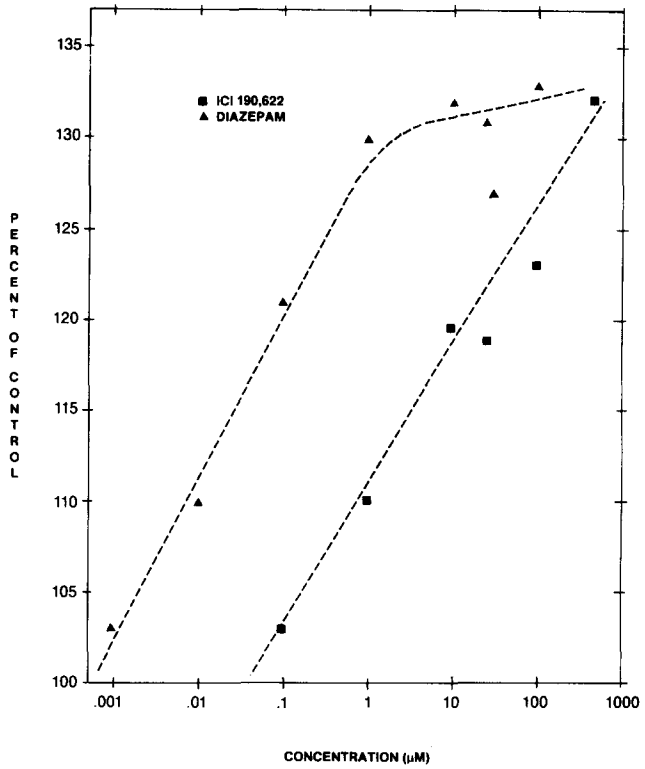


FIG. 3. The enhancement of specific [^{3}H]GABA binding to a membrane fraction of rat brain following incubation at 37°C was determined as described in the text. Each curve was compiled from at least three separate experiments with SEM less than 4%.

than the Type 2 receptor. In this respect, ICI 190,622 is more like CL 218872 [3] or zolpidem than the benzodiazepines.

It has been reported [16] that zopiclone and related compounds cause a reduction in [^{3}H]FLU binding at a site allosteric to the benzodiazepine binding site. The evidence for this conclusion was that, in the presence of zopiclone, there was a reduction in the apparent number of benzodiazepine binding sites with little change in the apparent affinity. To establish that ICI 190,622 was acting directly at the benzodiazepine binding site, and not at an allosteric site, similar experiments were performed. As expected for a competitive inhibitor, ICI 190,622 (20 nM) caused a decrease in the apparent affinity of [^{3}H]FLU (increase in K_D from 2.4 nM to 4.7 nM) for its binding site but no change in the number of sites (B_{max}). Therefore, ICI 190,622 appears to be acting competitively at the benzodiazepine receptor.

Agonist Properties of ICI 190,622

It is possible to distinguish benzodiazepine antagonists from the anxiolytic benzodiazepines (agonists) by several biochemical criteria. Specifically, GABA increases the affinity of anxiolytic benzodiazepines for their binding site [15,18]. However, the affinity of the benzodiazepine antagonists does not appear to be altered by GABA [2].

GABA (100 μM) caused an increase (1.3-fold) in the affinity of ICI 190,622 (Table 6). Similarly, a GABA shift greater than 1.0 was obtained with the other agonists (e.g., a 1.6-fold shift with diazepam). In contrast, the three benzodiazepine antagonists, Ro15-1788, BCCE, and CGS 8216, showed a GABA shift of 1.0 or less. These data suggest that ICI 190,622 has agonist-like properties.

It has been shown previously that anxiolytic benzodiazepines, as well as tracazolate and CL 218872, enhance GABA binding [8]. In contrast, benzodiazepine antagonists will reverse the benzodiazepine-induced enhancement of

GABA binding [9]. The effect of ICI 190,622 on this system was determined using the method of Meiners and Salama [9]. Like the anxiolytic benzodiazepines, ICI 190,622 was found to enhance GABA binding (Fig. 3). Furthermore, both Ro15-1788 (50 μM) and CGS 8216 (10 μM) were able to reverse the enhancement of GABA binding by ICI 190,622 (10 μM). These results strongly suggest that ICI 190,622 increases GABA binding by acting as an agonist at the benzodiazepine site.

Ex Vivo *Effects on [^{3}H]FLU Binding*

Although *in vitro* binding studies have yielded much information about the intrinsic affinity of ICI 190,622 at the benzodiazepine receptor, these results cannot shed any light on the metabolism, absorption, or duration of action of the compound. Insights into these aspects may be obtained through *ex vivo* experiments, whereby drug is administered to the animal and the level of the compound and/or its metabolites that can displace [^{3}H]FLU binding are then determined *in vitro*. Briefly, the rats were administered drug or vehicle and then decapitated at various times. The brains were dissected and frozen, and [^{3}H]FLU binding to a crude homogenate of the brain tissue was determined at a later time.

The *ex vivo* potency of ICI 190,622 in cerebellum was slightly greater than that of diazepam (IC_{50}=2.5 and 6 mg/kg, PO, respectively, Fig. 4) 1.0 hour after administration. Interestingly, the displacement of [^{3}H]FLU from the cerebral cortex by ICI 190,622 occurred at much higher doses (Fig. 4), confirming the selectivity for the Type 1 receptor noted *in vitro*.

SUMMARY AND CONCLUSION

ICI 190,622, a pyrazolopyridine, demonstrated potent anxiolytic activity in rats and monkeys. ICI 190,622 appears to be an "anxioselective" agent in that it exhibits only very weak, sedative activity, and its sedative liability ratio is considerably larger than that of benzodiazepine. In addition, its interactions with other CNS depressants (e.g., ethanol) is likely to be significantly less pronounced compared to the interactions of benzodiazepine with these agents. Furthermore, ICI 190,622 exhibited potent activity as an antagonist of both metrazole- and bicuculline-induced convulsions.

Neurochemically, ICI 190,622 is like the benzodiazepines in that it potently displaces [^{3}H]FLU from its binding sites in brain both *in vitro* and *ex vivo;* although ICI 190,622 was less potent than diazepam as a displacer *in vitro,* it was slightly more potent than diazepam *ex vivo* following oral administration. However, unlike diazepam, which is essentially equally effective as a displacer of binding from both Type 1 and Type 2 benzodiazepine sites, ICI 190,622 has a higher affinity for the Type 1 receptor *in vitro* and *ex vivo*. Thus, based upon pharmacological studies in animals, ICI 190,622 represents a novel non-benzodiazepine anxiolytic agent that appears to offer significant advantages over the benzodiazepines.

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