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The interaction of substituted benzamides with brain benzodiazepine binding sites *in vitro*

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1 The interaction of substituted benzamides with brain benzodiazepine (BDZ) binding sites was examined by their ability to displace [^{3}H]-flunitrazepam ([^{3}H]-FNM) from specific binding sites in bovine cortical membranes *in vitro*.

2 Clebopride, Delagrange 2674, Delagrange 2335 and BRL 20627 displayed concentration-dependent displacement of [^{3}H]-FNM with IC_{50} values of 73 nM, 132 nM, 7.7 μM and 5.9 μM, respectively. Other substituted benzamides including metoclopramide, sulpiride, tiapride, sultopride and cisapride were inactive at 10^{-5} M.

3 Inhibition by clebopride and Delagrange 2674 of [^{3}H]-FNM binding was apparently competitive and readily reversible.

4 In the presence of γ-aminobutyric acid (GABA), the ability of diazepam and Delagrange 2674 to displace [^{3}H]-Ro 15-1788 binding was increased 3.6 and 1.6 fold respectively, compared to the absence of GABA, while ethyl β-carboline-3-carboxylate (βCCE) and clebopride were less potent in the presence of GABA.

5 Diazepam was 30 fold less potent at displacing [^{3}H]-Ro 15-1788 in membranes that had been photoaffinity labelled with FNM than in control membranes, whereas the potency of βCCE did not differ. Clebopride and Delagrange 2674 showed a less than two fold loss of potency in photoaffinity labelled membranes.

6 The pattern of binding of clebopride and Delagrange 2674 in these *in vitro* tests is similar to that found previously with partial agonists or antagonists at BDZ binding sites.

7 Clebopride and Delagrange 2674 inhibited [^{3}H]-FNM binding with similar potency in rat cerebellar and hippocampal membranes, suggesting they have no selectivity for BDZ_1 and BDZ_2 binding sites.

8 Clebopride and Delagrange 2674 are structurally dissimilar to other BDZ ligands and represent another chemical structure to probe brain BDZ binding sites.

Introduction

Substituted benzamides are a series of analogues of the drug, metoclopramide, and include sulpiride, sultopride, tiapride and clebopride. They possess, to a greater or lesser extent, anti-emetic, anti-psychotic and anti-dyskinetic activity in man. Animal behavioural and biochemical studies indicate that these drugs are selective dopamine antagonists (Elliott *et al.*, 1977; Jenner & Marsden, 1979a,b; Roberts, 1982). Although they differ one from another, as a group they exhibit only part of the spectrum of biochemical and behavioural effects seen with typical neuroleptic agents, such as phenothiazines and thioxanthenes. In part this is a result of a selective interaction of substituted benzamides with dopamine D_2-receptors, since these compounds do not interact with dopamine D_1-receptors and in general do not affect adrenergic, 5-hydroxytryptamine, histamine or

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acetylcholine receptors (Jenner & Marsden, 1979a,b; Jenner *et al.*, 1982).

It has been demonstrated that two substituted benzamides, sulpiride and tiapride possess anxiolytic activity, clinically and in an experimental mouse model (Costall *et al.*, 1987). Since many anxiolytic drugs exert their effects by interacting with brain benzodiazepine (BDZ) binding sites, we have investigated whether substituted benzamides interact with brain BDZ binding sites *in vitro*.

Part of this work has been presented in preliminary form to the British Pharmacological Society (Chivers *et al.*, 1986).

Methods

Membrane preparations

Bovine brains were collected on ice from a local abattoir. Cerebral cortex was dissected and homogenized in 20 volumes of ice-cold 50 mM Tris-citrate pH 7.1 and centrifuged at 50,000 *g* for 10 min at 4°C. Homogenization and centrifugation were repeated two further times and the membranes stored in aliquots at −20°C. On the day of use, the membranes were thawed, homogenized and centrifuged 5 times and resuspended at the required concentration. Hippocampal and cerebellar membranes were prepared from male Wistar rats by the same method.

[^{3}H]-flunitrazepam binding

[^{3}H]-flunitrazepam ([^{3}H]-FNM, final concentration 0.5 nM for bovine cortex, 2 nM for rat hippocampus and cerebellum) was incubated with membrane (equivalent to 4 mg wet wt for bovine cortex and 6 mg wet wt for rat hippocampus and cerebellum) in 50 mM Tris-citrate pH 7.1 for 90 min at 0°C in the presence and absence of test drugs. In saturation binding experiments 8 concentrations (0.05–10 nM) of [^{3}H]-FNM were used.

Effect on γ-aminobutyric acid (GABA) on drug displacements

[^{3}H]-Ro 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-[^{3}H]-6-oxo-4H-imidazo-[1,5a][1,4]benzodiazepine-3-carboxylate) (1 nM) was incubated with membrane (equivalent to 4 mg wet wt of bovine cortex) in 50 mM Tris-citrate pH 7.1 for 60 min at 0°C. The ability of test drugs to inhibit [^{3}H]-Ro 15-1788 binding was compared concurrently in the presence and absence of 100 μM GABA and 200 mM NaCl.

Drug displacements in photoaffinity labelled membranes

Bovine cortical membranes were incubated for 90 min at 0°C in the presence of 10 nM FNM, exposed to u.v. irradiation for 15 min and washed 4 times with 20 volumes 50 mM Tris-citrate pH 7.1 to remove reversibly bound FNM. Displacement of [^{3}H]-Ro 15-1788 by test drugs was determined concurrently in photoaffinity labelled (PAL) and control membranes (treated identically except not exposed to u.v.).

Diazepam (a classical BDZ ligand with anxiolytic, sedative and anticonvulsant properties) and β-carboline-3-carboxylate ethyl ester (βCCE, a partial inverse agonist at BDZ binding sites, with anxiogenic and pro-convulsant properties) were used as reference compounds when studying the effect of GABA and photoaffinity labelling on drug displacements.

Membrane bound radioactivity was separated by filtration through Whatman GF/B filters under vacuum, by use of a Brandel cell harvester (Gaithersberg, USA). Filters were washed rapidly 4 times with 4 ml of ice-cold incubation buffer and radioactivity determined by liquid scintillation counting, at 40–45% efficiency. Specific binding was defined as the radioactivity displaceable by 2 μM clonazepam (a potent and selective ligand for central BDZ binding sites).

Results

Inhibition of [^{3}H]-FNM binding

In preliminary experiments, a number of substituted benzamides and structurally related compounds were tested for their ability to displace [^{3}H]-FNM from specific binding site in bovine cortical membranes. Most compounds, including metoclopramide, sulpiride, sultopride, tiapride and cisapride, were inactive at concentrations up to 10^{-5} M (Table 1). However, clebopride, Delagrange 2674 and 2335, and BRL 20627 (structures shown in Figure 1) displaced [^{3}H]-FNM in a concentration-dependent manner, with maximal displacement to the same level as clonazepam. IC_{50} values for clebopride and Delagrange 2674 were near to 100 nM while BRL 20627 and Delagrange 2335 were at least 50 fold weaker (Table 1). Slope coefficients for these four compounds, and for diazepam, were close to unity (Table 1). The two most active compounds, clebopride and Delagrange 2674 were studied further.

Clebopride and Delagrange 2674 inhibited [^{3}H]-FNM binding in a competitive manner, as did diazepam. Saturation binding of [^{3}H]-FNM, in the pre-

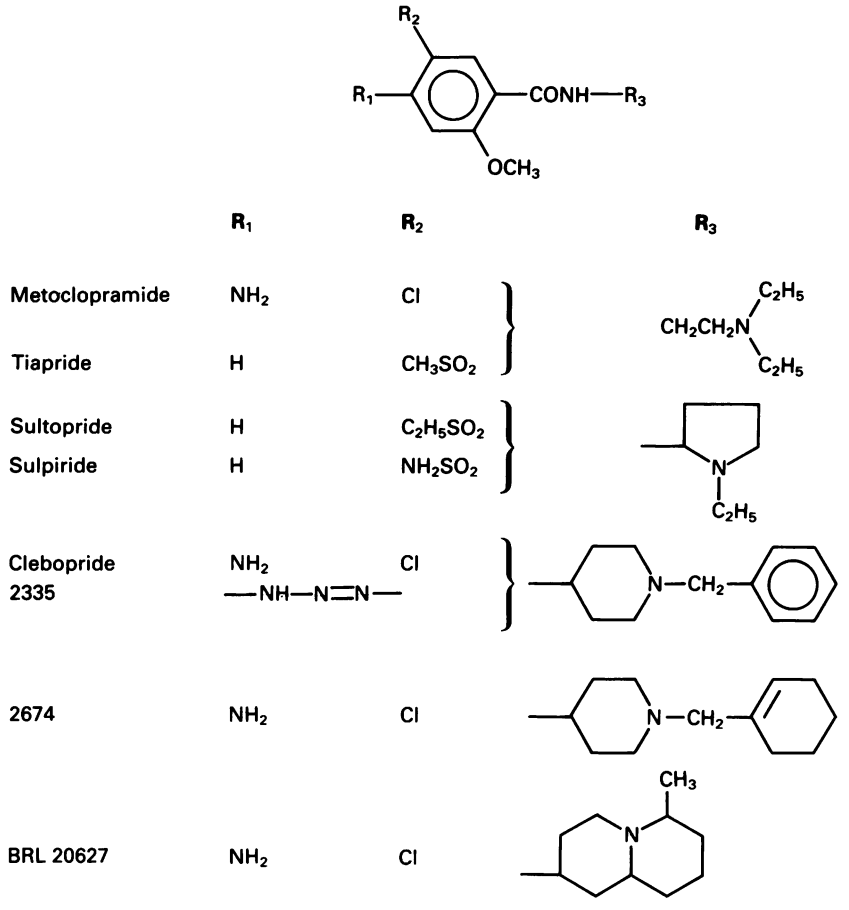

Figure 1 The structural formulae of some substituted benzamides currently under investigation.

sence of concentrations of drugs close to the determined IC_{50} values showed a decrease in the affinity, with no change in the maximal number of binding sites (Table 2).

Inhibition of [^{3}H]-FNM binding by clebopride, Delagrange 2674 and diazepam was readily reversible. Inhibition of binding to less than 50% of control values was restored to 83–93% of control values after a single washing and resuspension of the membrane and to 91–100% by a further wash (results not shown in detail).

Effects of GABA on drug displacements

The ability of diazepam to displace [^{3}H]-Ro 15–1788 binding was increased 3–4 fold in the presence of GABA (Table 3). In contrast βCCE had a lower displacing potency in the presence of GABA. Clebopride behaved similarly to βCCE in that it was a weaker displacer of [^{3}H]-Ro 15-1788 in the presence of GABA while Delagrange 2674 was slightly more potent in the presence of GABA (Table 3).

Drug displacements in photoaffinity labelled membranes

The ability of diazepam to displace [^{3}H]-Ro 15–1788 binding was reduced some 30 fold in photoaffinity labelled (PAL) membranes compared to control membranes, whilst the activity of βCCE was unchanged (Table 4). Clebopride and Delagrange 2674 were slightly less potent in PAL membranes, giving photoaffinity shifts greater than unity (Table 4).

Table 1 Displacement of [^{3}H]-flunitrazepam ([^{3}H]-FNM) from bovine cortical membranes

	IC_{50}	*Slope coefficient*
Diazepam	18 ± 4	0.97 ± 0.03
Clebopride	73 ± 11	1.12 ± 0.10
Delagrange 2674	132 ± 19	0.99 ± 0.08
BRL 20627	5900 ± 4000	0.96 ± 0.04
Delagrange 2335	7700 ± 2000	0.91 ± 0.03

The following compounds were inactive at 10^{-5} M in displacing [^{3}H]-FNM binding: sulpiride, metoclopramide, tiapride, sultopride, Delagrange 1433, 1511, DER 1813, 2093, 2304, 2308, 2366, 2691, 2706, 2711, 2735, 2738, 2762 (32% inhibition at 10^{-5} M), 2776, 2801, 2851, 2875 (Delagrange, France) BRL 20596, BRL 22594, BRL 27320 (Beechams, Harlow, UK) MD 240195, MD 790501 (Delalande, France) Cisapride (Janssen Pharmaceutica, Belgium) SL 74205 (LERS Synthelabo, France) FLA 870, FLB 131 (Astra, Sweden) YM 09151-2 (Yamanouchi, Japan).

[^{3}H]-FNM binding (0.5 nM) was determined in the absence and presence of displacing drugs (12 concentrations). IC_{50} (nM) values are the drug concentration required to inhibit specific binding by 50% and were determined graphically. Slope coefficients (pseudo Hill plots) were determined from plots of log $[\%B_{max}/100 - (\%B_{max})]$ against log displacer, where binding in the presence of displacer is expressed as a % of the maximal binding in the absence of displacer. Values are mean ± s.e.mean of 3–4 independent determinations.

Brain regional selectivity of drug inhibition of [^{3}H]-FNM binding

Diazepam inhibited the binding of [^{3}H]-FNM with comparable potencies in rat hippocampal and cerebellar membranes, whereas βCCE was some 6 fold more potent in cerebellar than hippocampal membranes (Table 5). Clebopride gave an IC_{50} ratio in hippocampal and cerebellar membranes close to unity and comparable to diazepam. Delagrange 2674 was somewhat more potent in cerebellar than hippocampal membranes, but the difference was less than 2 fold (Table 5).

Table 2 Saturation of binding of [^{3}H]-flunitrazepam ([^{3}H]-FNM) in bovine cortical membranes

	K_D (nM)	B_{max} (pmol g^{-1} tissue)
Control	1.2 ± 0.1	13.4 ± 0.8
Diazepam	3.2 ± 0.4	14.3 ± 1.8
Clebopride	3.0 ± 0.3	14.8 ± 1.9
Delagrange 2674	2.6 ± 0.2	13.7 ± 1.2

Binding of [^{3}H]-FNM (8 concentrations, 0.05–10 nM) was determined in the presence of drug vehicle, 20 nM diazepam, 80 nM clebopride, and 140 nM Delagrange 2674. Specific binding was converted to Scatchard plots and the equilibrium dissociation constants (K_D) and maximal number of binding sites (B_{max}) determined by linear regression analysis. Values are mean ± s.e.mean.

Discussion

We have demonstrated an interaction of 4 substituted benzamides with brain BDZ binding sites *in vitro*. Clebopride and Delagrange 2674 had IC_{50} values for displacement of [^{3}H]-FNM within the concentration-range of many clinically active BDZs whereas BRL 20627 and Delagrange 2335 had IC_{50} values in the low micromolar range and were less potent at displacing [^{3}H]-FNM than chlordiazepoxide (Speth *et al.*, 1980). The four substituted benzamides displaced to the same maximal extent as clonazepam, had slope coefficients close to unity and their inhibition was readily reversed on removal of the drug by washing. Saturation binding of [^{3}H]-FNM in the presence of clebopride and Delagrange 2674, as well as diazepam, increased the K_D without altering the maximal number of binding sites labelled. These results are compatible with a com-

Table 3 Effect of γ-aminobutyric acid (GABA) on the displacement of [^{3}H]-Ro 15-1788 from bovine cortical membranes

	IC_{50} (nM) *−GABA*	*+GABA*	*GABA shift*
Diazepam	58.3 ± 6.0	17.3 ± 3.9	3.57 ± 0.52
βCCE	6.0 ± 1.8	7.5 ± 2.3	0.80 ± 0.05
Clebopride	122 ± 39	168 ± 54	0.73 ± 0.05
Delagrange 2674	237 ± 7	150 ± 2	1.58 ± 0.05

Values are means ± s.e.mean (n = 3–4). GABA shift is defined as the ratio of IC_{50} in the presence of GABA to the IC_{50} in the absence of GABA. βCCE = ethyl β-carboline-3-carboxylate.

Table 4 Displacement of [^{3}H]-Ro 15-1788 in photoaffinity labelled (PAL) and control bovine cortical membranes

	IC_{50} (nM)		
	PAL	*Control*	*Photoaffinity shift*
Diazepam	2417 ± 167	82 ± 4	30 ± 4
βCCE	15.8 ± 5.1	16.0 ± 4.9	0.98 ± 0.02
Clebopride	223 ± 48	150 ± 23	1.46 ± 0.09
Delagrange 2674	317 ± 44	247 ± 32	1.30 ± 0.17

Values are means ± s.e.mean (n = 3–4). Photoaffinity shift is defined as the ratio of the IC_{50} in PAL membranes to the IC_{50} in control membranes. βCCE = ethyl β-carboline-3-carboxylate.

petitive interaction of clebopride and Delagrange 2674 with BDZs for the same binding sites.

Drugs of very diverse chemical structures have been shown to interact with brain BDZ binding sites and to produce a spectrum of pharmacological effects ranging from full agonists (anxiolytic, hypnotic, anticonvulsant and muscle relaxant) to inverse agonists (anxiogenic and convulsant), with antagonists lacking intrinsic pharmacological activity but preventing or blocking the effects of agonists and inverse agonists (reviewed by Braestrup *et al.*, 1984). Full and partial BDZ agonists, as well as many 1,4 BDZs, include CL 218,872 (triazolopyridazine), PK 8165 and PK 9084 (quinoline derivatives), suriclone (cyclopyrrolone), triazolam and alprazolam (triazolobenzodiazepines) and zolpidem (imidazopyridine) (Lippa *et al.*, 1979; Le Fur *et al.*, 1981; Blanchard & Julon, 1983; Sethy *et al.*, 1983; Arbilla *et al.*, 1985). Inverse agonists include various esters of β-carboline-3-carboxylate (Braestrup *et al.*, 1980; Oakley & Jones, 1980). Various substituted benzamides must now be added to the growing list of diverse chemical structures which interact with brain BDZ binding sites.

To define in more detail the nature of the interaction between substituted benzamides and brain BDZ binding sites, we have applied two *in vitro* tests which discriminate between BDZ ligands with different pharmacological activities. Firstly, the ability of agonists to displace specific [^{3}H]-Ro 15–1788 binding (the prototype BDZ antagonist) is increased in the presence of GABA compared to the absence, giving so called 'GABA shifts' greater than unity, whereas inverse agonists have reduced affinity in the presence of GABA (GABA shift less than 1) and antagonists are unaffected (GABA shift = 1) (Ehlert *et al.*, 1981; Möhler & Richards, 1981; Skolnick *et al.*, 1982). Secondly, unlabelled FNM binds irreversibly to a proportion of BDZ binding sites when exposed to u.v. light (Möhler *et al.*, 1980). The non-photolabelled sites retain their affinity for binding [^{3}H]-Ro 15-1788 and antagonists and inverse agonists have respectively identical or slightly reduced abilities to displace [^{3}H]-Ro 15–1788 (i.e. they have so called photoaffinity shifts of 1 and slightly less than 1, respectively). In contrast, agonists have very much reduced abilities to displace [^{3}H]-Ro 15-1788 binding in PAL membranes (photoaffinity shifts > 1) (Karobath & Supavilai, 1982; Möhler, 1982).

The GABA shift for diazepam was 3–4 and for βCCE was less than 1, agreeing broadly with published values obtained with rat membranes (Möhler & Richards, 1981; Skolnick *et al.*, 1982; Stapelton *et al.*, 1982). Clebopride gave a GABA shift similar to βCCE, suggesting inverse agonist or antagonist activity, while Delagrange 2674 gave a shift slightly greater than unity. This may indicate weak agonist activity since most other non-BDZ

Table 5 Displacement of [^{3}H]-flunitrazepam ([^{3}H]-FNM) binding in rat cerebellum and hippocampus

	IC_{50} (nM)		Ratio $\left\{\frac{IC_{50}\ Hippocampus}{IC_{50}\ Cerebellum}\right.$
	Hippocampus	*Cerebellum*	
Diazepam	26.4 ± 3.9	21.4 ± 2.6	1.22 ± 0.08
βCCE	5.5 ± 0.9	0.95 ± 0.13	5.7 ± 0.3
Clebopride	131 ± 20	103 ± 15	1.26 ± 0.01
Delagrange 2674	338 ± 43	198 ± 35	1.75 ± 0.17

Specific binding of [^{3}H]-FNM (2 nM) was determined concurrently in hippocampal and cerebellar membranes in the presence and absence of displacing drug (9 concentrations). Values are means ± s.e.mean (n = 3–4). βCCE = ethyl β-carboline-3-carboxylate.

drugs with agonist or partial agonist activity have GABA shifts less than the classical 1,4 BDZs e.g. CL 218,872, PK 8165, ZK 91296, alprazolam and triazolam (Skolnick *et al.*, 1982; Petersen *et al.*, 1984; Clow *et al.*, 1985), although zolpidem seems to be an exception (Arbilla *et al.*, 1985).

Photoaffinity shifts for diazepam and βCCE were comparable with previous reports (Karobath & Supavilai, 1982; Möhler, 1982). Clebopride and Delagrange 2674 gave photoaffinity shifts slightly greater than 1, suggesting weak agonist or antagonist activity. Most non-BDZ with agonist or partial agonist activity at BDZ binding sites have photoaffinity shifts greater than 2, e.g. zopiclone, CL 218,872, PK 9084 (Möhler, 1982; Karobath & Supavilai, 1982), although the partial agonist ZK 91296 has a photoaffinity shift of slightly less than 2 (Petersen *et al.*, 1984).

Thus clebopride and Delagrange 2674 clearly differ from full BDZ agonists, such as diazepam in these *in vitro* binding studies. Their profile in these tests is similar to those previously reported with partial agonists and antagonists at BDZ binding sites. Clearly behavioural and electrophysical experiments will be required to test these predictions.

Several drugs that interact with brain BDZ binding sites differ in their affinity in different brain regions. For example quazepam, halazepam, CL 218,872 and βCCE are more potent at displacing [^{3}H]-BDZ ligands in the rat cerebellum than the rat hippocampus (Braestrup & Nielsen, 1981; Stapleton *et al.*, 1982; Sieghart, 1983; Iorio *et al.*, 1984; Sieghart & Schuster, 1984). The proposed explanation is that the above drugs have a higher affinity for the so-called BDZ_1 subset of binding sites, which is the predominant (or only) type in the cerebellum whereas the hippocampus contains both BDZ_1 and BDZ_2 subtypes (Braestrup & Nielsen, 1981). The present results confirm the greater displacing potency of βCCE in the cerebellum than hippocampus, whereas the potency of clebopride and Delagrange 2674, as well as diazepam and most classical 1,4 BDZs (Sieghart & Schuster, 1984) differed little in the two brain regions. Thus clebopride and Delagrange 2674 appear to have no selectivity for BDZ_1 and BDZ_2 subtypes of binding sites.

The present results indicate that the binding of substituted benzamides to BDZ receptors is highly structure specific, and the nature of aromatic substituents, the basic side-chain and N-substituents all seem to play a critical role. Thus, the existence of a 4-amino-5-chloro substitution pattern on the aromatic ring is a major prerequisite for binding. In addition, only when the side-chain is a 4-piperidinyl ring does affinity exist. A side-chain with only two carbon atoms separating the amide nitrogen and the basic nitrogen apparently leads to a total lack of affinity (e.g. metoclopramide). Another condition for binding may be a bulky and flexible N-substituent. As a result of these limitations, only clebopride and Delagrange 2674 of the compounds tested display noteworthy affinity. However, the number of compounds currently investigated is limited and does not allow unambiguous assessment of the individual influence of each structural feature.

While a considerable variety of basic chemical structures have been shown to bind to BDZ receptors (Haefely *et al.*, 1985), the binding of clebopride and Delagrange 2674 is a most unexpected finding. Indeed, the chemical structure of these atypical neuroleptics shows little apparent similarity with that of benzodiazepines, triazolo- and imidazobenzodiazepines, cyclopyrrolones, phenyl- and pyrazoloquinolines, triazolopyridazines, β-carbolines and other hitherto recognized BDZ ligands. The very limited number of active benzamides precludes any rationalization in terms of structure-binding relationships.

The interaction of substituted benzamides with brain BDZ binding sites does not appear to be related to their antagonist activity at dopamine D_2-receptors. It is interesting to note that BRL 20596, which is the anilide derivative of clebopride where the amide bond has been reversed, retains potent central dopamine receptor antagonist properties (Blaney *et al.*, 1983), but is inactive at BDZ binding sites (Table 1).

The reported anxiolytic properties of sulpiride and tiapride in a mouse model (Costall *et al.*, 1987) would appear not to result from an interaction with brain BDZ sites, since neither drug was able to displace BDZ binding *in vitro* at 10^{-5} M. Alternative sites of action (for example 5-HT_{1A} or barbiturate binding sites) may be responsible for their putative anxiolytic properties.

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References

ARBILLA, S., DEPOORTERE, H., GEORGE, P. & LANGER, S.Z. (1985). Pharmacological profile of the imidazopyridine zolpidem at benzodiazepine receptors and electrocorticogram in rats. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **330**, 248–251.

BLANCHARD, J-C. & JULOU, L. (1983). Suriclone: a new

cyclopyrrolone derivative recognizing receptors labelled by benzodiazepines in rat hippocampus and cerebellum. *J. Neurochem.*, **40**, 601–607.

BLANEY, F.E., CLARK, M.S.G., GARDNER, D.V., HADLEY, M.S., MIDDLETON, D. & WHITE, T.J. (1983). Anilides related to substituted benzamides. Potential antipsychotic activity of N-(4-amino-5-chloro-2-methoxyphenyl)-1-(phenylmethyl)-4-piperidinecarboxamide. *J. Med. Chem.*, **26**, 1747–1752.

BRAESTRUP, C. & NIELSEN, M. (1981). GABA reduces binding of 3H-methyl β-carboline-3-carboxylate to brain benzodiazepine receptors. *Nature*, **294**, 472–474.

BRAESTRUP, C., HONORÉ, T., NIELSEN, M., PETERSEN, E.N. & JENSEN, L.H. (1984). Ligands for benzodiazepine receptors with positive and negative efficacy. *Biochem. Pharmacol.*, **33**, 859–862.

CHIVERS, J.K., HORTON, R.W., JENNER, P.G., LOWTHER, S., MARSDEN, C.D. & TESTA, B. (1987). Interaction of the substituted benzamide, clebopride with brain benzodiazepine binding sites. *Br. J. Pharmacol.*, **90**, 123P.

COSTALL, B., HENDRIE, C.A., KELLY, M.E. & NAYLOR, R.J. (1987). Actions of sulpiride and tiapride in a simple model of anxiety in mice. *Neuropharmacol.*, **26**, 195–200.

CLOW, A., GLOVER, V. & SANDLER, M. (1985). Triazolam, an anomalous benzodiazepine ligand: *in vitro* characterization of alprazolam and triazolam binding. *J. Neurochem.*, **45**, 621–625.

EHLERT, F.J., ROESKE, W.R. & YAMAMURA, H.I. (1981). Multiple benzodiazepine receptors and their regulation by γ-aminobutyric acid. *Life Sci.*, **29**, 235–248.

ELLIOTT, P.N.C., JENNER, P., HUIZING, G., MARSDEN, C.D. & MILLER, R. (1977). Substituted benzamides as central dopamine antagonists in rodents. *Neuropharmacol.*, **16**, 333–342.

HAEFELY, W., KYBURZ, E., GERECKE, M. & MÖHLER, H. (1985). Recent advances in the molecular pharmacology of benzodiazepine receptors and in the structure-activity relationships of their agonists and antagonists. In *Advances in Drug Research*. ed. Testa, B. Vol. 15, pp. 165–322. London: Academic Press.

IORIO, L.C., BARNETT, A. & BILLARD, W. (1984). Selective affinity of 1-N-trifluoroethyl benzodiazepines for cerebellar type 1 receptor sites. *Life Sci.*, **35**, 105–113.

JENNER, P. & MARSDEN, C.D. (1979a). The substituted benzamides—a novel class of dopamine antagonists. *Life Sci.*, **25**, 479–486.

JENNER, P. & MARSDEN, C.D. (1979b). The mechanism of action of substituted benzamide drugs. In *Sulpiride and Other Benzamides*. ed. Spano, P.F., Trabucchi, M., Corsini, G.U. & Gessa, G.L. pp. 119–147. Milan: Italian Brain Research Foundation Press.

JENNER, P., THEODOROU, A. & MARSDEN, C.D. (1982). Specific receptors for substituted benzamide drugs in brain. In *The Benzamides: Pharmacology, Neurobiology and Clinical Aspects*. ed. Rotrosen, J. & Stanley, M. pp. 109–141. New York: Raven Press.

KAROBATH, M. & SUPAVILAI, P. (1982). Distinction of benzodiazepine agonists from antagonists by photoaffinity labelling of benzodiazepine receptors *in vitro*. *Neurosci. Letts.*, **31**, 65–69.

LE FUR, G., MIZOULE, J., BURGEVIN, M.C., FERRIS, O., HEAULME, M., GAUTHIER, A., GUÉRÉMY, G. & UZAN, A. (1981). Multiple benzodiazepine receptors: Evidence of a dissociation between anticonflict and anticonvulsant properties by PK 8165 and PK 9804. (two quinoline derivatives). *Life Sci.*, **28**, 1439–1449.

LIPPA, A.S., COUPET, J., GREENBLATT, E.N., KLEPNER, C.A. & 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.

MÖHLER, H. (1982). Benzodiazepine receptors: differential interaction of benzodiazepine agonists and antagonists after photoaffinity labelling with flunitrazepam. *Eur. J. Pharmacol.*, **80**, 435–436.

MÖHLER, H. & RICHARDS, J.G. (1981). Agonist and antagonist benzodiazepine receptor interaction *in vitro*. *Nature*, **294**, 763–765.

MÖHLER, H., BATTERSBY, M.K. & RICHARDS, J.G. (1980). Benzodiazepine receptor protein identified and visualized in brain tissue by a photoaffinity label. *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 1666–1670.

OAKLEY, N.R. & JONES, B.J. (1980). Proconvulsant and diazepam-reversing effects of ethyl β-carboline-3-carboxylate. *Eur. J. Pharmacol.*, **68**, 381–382.

PETERSEN, E.N., JENSEN, L.H., HONORÉ, T., BRAESTRUP, C., KEHR, W., STEPHENS, D.N., WACHTEL, H., SEIDELMAN, D. & SCHMEICHEN, R. (1984). ZK 912961, a partial agonist at benzodiazepine receptors. *Psychopharmacol.*, **83**, 240–248.

ROBERTS, D.J. (1982). The pharmacological basis of the therapeutic activity of clebopride and related substituted benzamides. *Curr. Therap. Res.*, **31**, Supp. No. 1S, S1–S44.

SETHY, V.H., RUSSEL, R.R. & DAENZER, C.I. (1983). Interaction of triazolobenzodiazepines with benzodiazepine receptors. *J. Pharm. Pharmacol.*, **35**, 524–526.

SIEGHART, W. (1983). Several new benzodiazepines selectively interact with a benzodiazepine receptor subtype. *Neurosci. Letts.*, **38**, 73–78.

SIEGHART, W. & SCHUSTER, A. (1984). Affinity of various ligands for benzodiazepine receptors in rat cerebellum and hippocampus. *Biochem. Pharmacol.*, **33**, 4033–4038.

SKOLNICK, P., SCHWERI, M.M., WILLIAMS, E.F., MONCADA, V.Y. & PAUL, S.M. (1982). An *in vitro* binding assay which differentiates benzodiazepine "agonists" and "antagonists". *Eur. J. Pharmacol.*, **78**, 133–136.

SPETH, R.C., JOHNSON, R.W., REGAN, J., REISINE, T., KOBAYASHI, R.M., BRESOLIN, N., ROESKE, W.R. & YAMAMURA, H.I. (1980). The benzodiazepine receptor of mammalian brain. *Fed. Proc.*, **39**, 3032–3038.

STAPLETON, S.R., PRESTWICH, S.A. & HORTON, R.W. (1982). Regional heterogeneity of benzodiazepine binding sites in rat brain. *Eur. J. Pharmacol.*, **84**, 221–224.

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