Imidazo[1,2-*b*]pyridazines: Syntheses and Interaction with Central and Peripheral-Type (Mitochondrial) Benzodiazepine Receptors

Gordon B. Barlin

Division of Neuroscience, John Curtin School of Medical Research, Australian National University, GPO Box 334, Canberra ACT 2601, Australia

The fundamental chemistry of pyridazines, the syntheses of substituted imidazo[1,2-*b*]pyridazines (**1**) (and some related compounds) and the interaction of the products with central benzodiazepine receptors (CBR) and peripheral-type (mitochondrial) benzodiazepine receptors (PBR) are described.

Some of these imidazo[1,2-*b*]pyridazines had high selective affinity for the central benzodiazepine receptors and others had high selectivity for the peripheral-type (mitochondrial) benzodiazepine receptors. The results of structure-activity studies and molecular modeling will be reported.

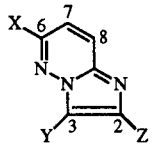
In vivo tests of some compounds which interacted strongly with the central benzodiazepine receptors revealed reasonably potent anticonvulsant/anticonflict activity, and some of those which bind selectively to the peripheral-type (mitochondrial) benzodiazepine receptors are being examined as possible radiopharmaceuticals for imaging of tumors (and other disease states).

J. Heterocyclic Chem., **35**, 1205 (1998).

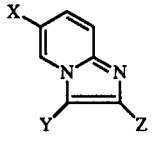
Introduction.

The pharmacological activities of some substituted pyridazines have been described in an excellent review, in two parts [1,2] by Professor Heinisch, which covers the literature for the periods 1975-1988 and 1975-1990. Vigorous research activity with substituted pyridazines has continued. Some examples from numerous recent publications include reports of anticonvulsant properties amongst benzylpyridazine derivatives [3], peripherally acting analgesic 3-arylpiperazinyl-5-benzylpyridazines [4], cardiotonic activity of the optically active pyridazin-3(2*H*)-one KF15232 [5], the determination of the structure of himastatin[6], an antitumor antibiotic which contains a reduced pyridazine ring [6], γ-aminobutyric acid-A (GABA-A) agonists from the condensation of muscimol or thiomuscimol with aminopyridazines [7], and antihypertensive and α-adrenoceptor activity of novel 2-aminoalkylpyridazin-3(2*H*)-ones [8]. Other recent reports for imidazo[1,2-*b*]pyridazines include antitumor carbon-bridged imidazopyridazine carbamates [9], analgesic imidazo[1,2-*b*]pyridazine-2-acetic acid derivatives [10] and a new class of angiotension II receptor antagonists bearing a substituted imidazo[1,2-*b*]pyridazine moiety [11].

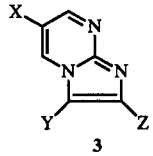
This communication summarizes the current situation relating to the synthesis of some 2,3,6-trisubstituted imidazo[1,2-*b*]pyridazines **1** and studies of their interaction with central benzodiazepine receptors (isolated from rat forebrain) and peripheral-type (mitochondrial) benzodiazepine receptors (isolated from rat kidney membrane), and of some *in vivo* studies in rats and mice. Some comparative studies have been made with analogously substituted imidazo[1,2-*a*]pyridines **2**, imidazo[1,2-*a*]pyrimidines **3** and imidazo[1,2-*a*]pyrazines **4**.



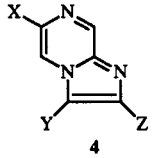
1



2



3



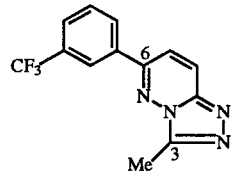
4

Biological activity in imidazo[1,2-*b*]pyridazines was first reported by Nitta, Yoneda and Otaka [12-15] at the Chugai Pharmaceutical Company with a series of patent applications [12-15] in 1963 which claimed analgesic, sedative and antispasmodic activity, and as an inhibitor of central nerves. Also Pfizer & Co., in a patent application [16] in 1966, claimed antihypertensive activity for a series of substituted imidazo[1,2-*b*]pyridazines.

We became involved in imidazo[1,2-*b*]pyridazine chemistry as a flow on from our study of the fundamental chemistry of pyridazines and certain ring closure reactions of aminopyridazines. We observed that when 3-amino-4-methylaminopyridazine was condensed with pyruvaldehyde dimethyl acetal in methanolic hydrogen chloride [17] it gave 3-methoxy-2-methyl-8-methylaminoimidazo[1,2-*b*]pyridazine as shown by X-ray analysis [17]; and 3-amino-6-chloropyridazine with phenylglyoxal in ethanol containing hydrochloric acid gave 6-chloro-2-phenylimidazo[1,2-*b*]pyridazin-3(5*H*)-one which was then methylated (with diazomethane) to 6-chloro-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (1, X = Cl, Y = OMe, Z = Ph) [18].

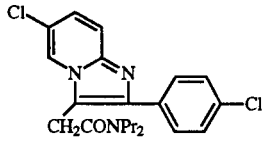
Subsequent biological testing [19] revealed that the last mentioned compound was capable of displacing [^{3}H]diazepam from rat brain membrane; and hence our continued interest in substituted imidazo[1,2-*b*]pyridazines. Our aim was to develop a compound (or compounds) with more selective pharmacological actions than the benzodiazepines.

In the closely related 1,2,4-triazolo[4,3-*b*]pyridazine ring system the compound CL 218872, **5**, [20] has anticonflict and anticonvulsant activities in animals and was found to produce sedation and myorelaxant effects at doses much higher than the anxiolytic doses [21,22]. The interaction of CL 218872 with benzodiazepine receptors has been examined extensively and it has been shown to be a partial agonist at individual receptor combinations [23,24].

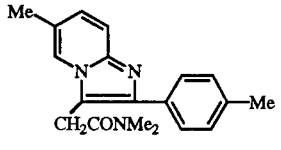


5 (CL 218872)

The imidazo[1,2-*a*]pyridines, alpidem **6** [25] [a ligand of both the central benzodiazepine receptor and the peripheral-type (mitochondrial) benzodiazepine receptor] has sedative and anxiolytic properties, and zolpidem **7** [25] (a selective ligand for the central benzodiazepine receptor) is marketed as an hypnotic. Both alpidem and zolpidem have higher affinity for benzodiazepine-1 than for benzodiazepine-2 receptors [23]; and their interaction with various receptor types has been reported [26].



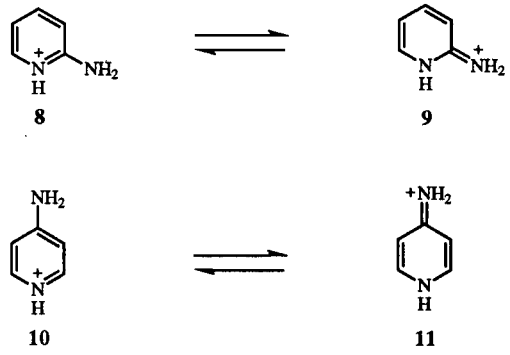
6 (Alpidem)



7 (Zolpidem)

Some Properties of Pyridazines and Syntheses of Imidazo[1,2-*b*]pyridazines.

Pyridazine (pK_a 2.33) [27] is a weaker base than pyridine (pK_a 5.23) [27] because of the adjacent competing basic nitrogen atoms in the former and 3-amino- and 4-amino-pyridazine (pK_as 5.19 [27] and 6.69 [28] respectively) are weaker bases than the corresponding aminopyridine (2-amino-, pK_a 6.86 [27]; 3-amino, pK_a 5.98 [27]; 4-amino-, pK_a 9.17 [27] in which the enhanced basicity of the 2- and 4-aminopyridines is due to resonance stabilization of the cation *e.g.* **8** ↔ **9**, and **10** ↔ **11** respectively). Imidazo[1,2-*b*]pyridazine would also be expected to be a weaker base than imidazo[1,2-*a*]pyridine.



8 **9**

10 **11**

Quaternization of 4-aminopyridazine with methyl iodide has been shown to give the 1- and 2-methiodides in the ratio 6:1 [28]; the higher proportion of the 1-methyl isomer is probably associated with the enhanced basicity of N-1 (relative to N-2). No methylation was detected at the extranuclear amino group. The identity of the 1-methyl compound was determined by its ready hydrolyses with hydroxide ion to 1-methylpyridazin-4(1*H*)-one.

Methylation of 3-aminopyridazine with methyl iodide however gave 3-amino-1-methylpyridazium iodide and its 2-methyl isomer in the ratio 4:1 [28]. No methylation was observed at the 3-amino group. The lower proportion of the 2-methyl compound is probably due to some steric hindrance at N-2 which favors methylation at N-1 rather than the more basic N-2. Hydrolysis of 3-amino-2-methylpyridazinium iodide with hydroxide ions readily gave 2-methylpyridazin-3(2*H*)-one [28]. The ease of the hydrolyses described above is consistent with kinetic data for hydrolyses of aminopyridine methiodides [29]. Some kinetic data are available concerning the nucleophilic replacement of substituents in the pyridazine ring. Hill and Krause [30] investigated the reactivity of 3-chloro- and 3,6-dichloropyridazines towards methoxide ions in methanol at 25.4° and obtained rate constants of 5.4 x 10^{-5}

and 2.8 x 10^{-3} l mol^{-1} sec^{-1} respectively, which clearly established the superior reactivity of the 3,6-dichloro compound. Under similar conditions 2-chloropyridine did not react appreciably [30].

Barlin and co-workers [31] have shown that the methylsulphonyl group in the 3- and 4-position of pyridazine is readily replaced by methoxide ions in methanol. The relevant rate constants (k_2) are as follows: 3-SO_2Me, 9.71 x 10^{-3} l mol^{-1} sec^{-1} (30.2°); 4-SO_2Me, 121 x 10^{-3} l mol^{-1} sec^{-1} (30.3°). In comparison to the reactivity of 3-chloropyridazine with methoxide ions in methanol [30], the 3-methylsulphonyl compound was *ca* 90 times more reactive, principally due to the lower energy of activation. The methylsulphinyl-compounds (MeSO-) showed reactivity similar to the methylsulphonyl compounds, whereas the methylthio compounds were significantly less reactive [32]. The comparative reactivity data for reactions with methoxide ions at 30° were: 3-SO_2Me/3-SMe = 3.71 x 10^5 and 4-SO_2Me/4-SMe = 4.76 x 10^4.

It has also been established [33], in other ring systems, that the trimethylammonio group is readily replaced by nucleophiles [33]. For example, pyrimidin-2-yltrimethylammonium chloride in reactions with hydroxide ions in water at 20° has been found to be *ca* 700 times more reactive than 2-chloropyrimidine and *ca* 5 times less reactive than 2-methylsulphonylpyrimidine [33].

In π-deficient nitrogen heterocycles the general order for nucleophilic replacement of leaving groups is as follows:

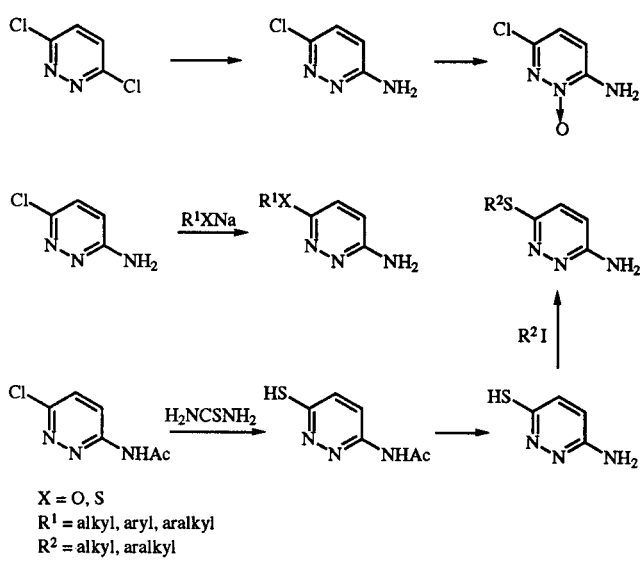
$$SMe < Cl < N^+Me_3 < SOMe \approx SO_2Me$$

The ease of replacement of substituents from π-deficient nitrogen heterocycles is also significantly influenced by quaternization (and protonation) (see the methylation of aminopyridazines and hydrolysis of the products discussed above), and by *N*-oxide formation. For example, 4-methylsulphonylpyridine methiodide is 7.3 x 10^8-fold more reactive than 4-methylsulphonylpyridine in reactions with hydroxide ions (in water) at 20° [34]; and in the pyridine [29] and the quinoline series [35] the enhancement of reactivity on quaternization in reactions with hydroxide ions is significantly greater for substituents at the α-position than for those at the γ-position [29,35]. The effect of the *N*-oxide group is shown in the rate of reaction of 2-chloropyridine *N*-oxide with piperidine in methanol (at 80°) [36] which is *ca* 10^3 times greater than that for 2-chloropyridine with piperidine in ethanol [37,38].

The methods for syntheses of the compounds required in this study were significantly influenced by our observations that 6-chloro-3-methoxy-2-phenylimidazo[1,2-*b*]-pyridazine with a) methoxide ions [39] in refluxing methanol for 5 hours gave 8% of the corresponding 6-methoxy compound, b) aqueous sodium methanethiolate [40] at 130-140° for 5 hours gave 6% of the 6-methylthio compound and c) aqueous sodium benzyloxide at 140° for 14 hours gave no 6-benzyloxy compound. Also the 6-chloro-substituent in 3-amino-6-chloropyridazine was not easily replaced by unchanged nucleophiles.

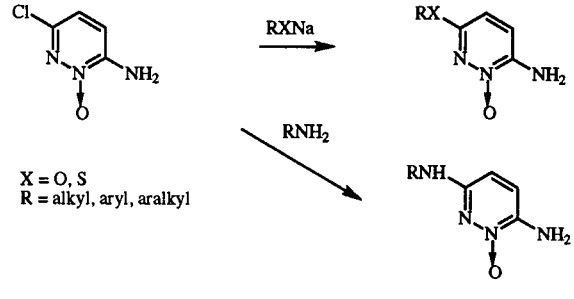
Our methods of syntheses generally commenced from 3,6-dichloropyridazine (obtained by chlorination [41] of 3,6-dihydroxypyridazine) through 3-amino-6-chloropyridazine [42] and 3-amino-6-chloropyridazine 2-oxide [43], and the substituent ultimately required at the 6-position of the imidazo[1,2-*b*]pyridazine was inserted into the 6-position of the pyridazine before cyclization to the bicyclic system. Some such transformations are set out in Schemes 1 and 2. Substituted 3-alkoxyimidazo[1,2-*b*]pyridazines

Scheme 1



X = O, S
R^1 = alkyl, aryl, aralkyl
R^2 = alkyl, aralkyl

Scheme 2

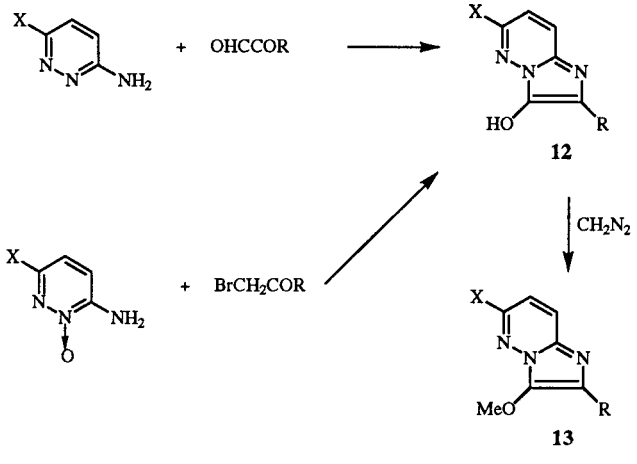


X = O, S
R = alkyl, aryl, aralkyl

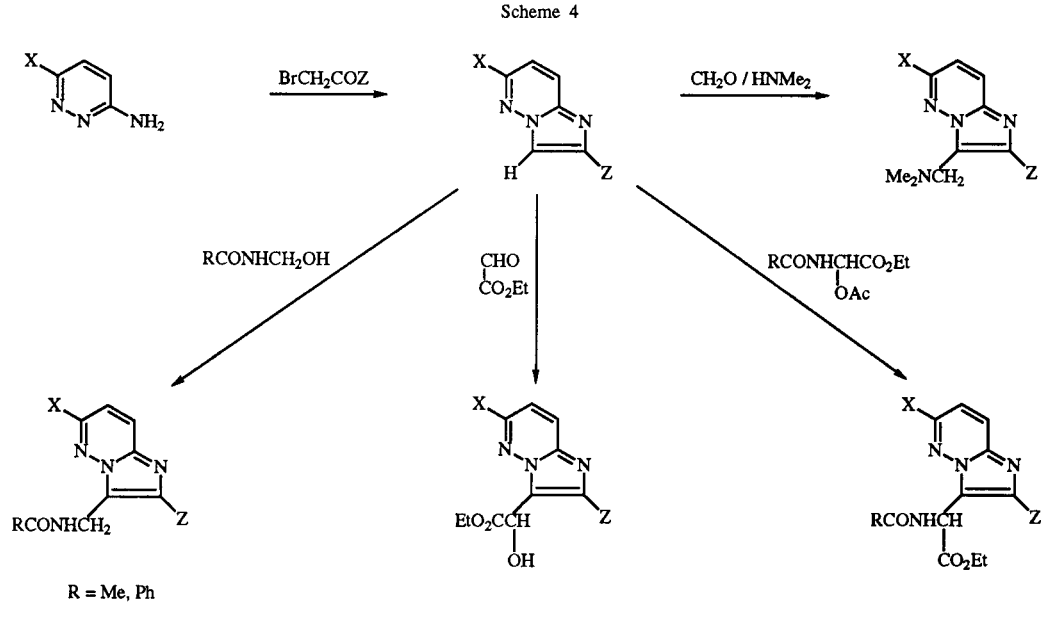
were prepared by two methods; from 3-aminopyridazines and from 3-aminopyridazine 2-oxides. Thus ring closures of the 3-amino-6-substituted pyridazines with glyoxals in ethanol containing aqueous hydrochloric acid gave the imidazo[1,2-*b*]pyridazine **12** (Scheme 3) which was methylated with diazomethane to the 3-methoxyimidazo-[1,2-*b*]pyridazine **13**. The same intermediate compound **12** could also be prepared from the corresponding 3-amino-6-substituted pyridazine 2-oxide with the appropriate bromoacetyl compound as shown in Scheme 3. No isomeric products were isolated from these diazomethane methylations except in the special case of 3-amino-6-hydroxypyridazine with *p*-tolylglyoxal followed by methylation of the product with diazomethane which gave both 3,6-dimethoxy-2-*p*-tolylimidazo[1,2-*b*]pyridazine and 3-methoxy-5-methyl-2-*p*-tolylimidazo[1,2-*b*]pyridazin-6(5*H*)-one.

The 3-unsubstituted imidazo[1,2-*b*]pyridazines **1** (Y = H) required (as intermediates) in this work were prepared from the relevant 3-amino-6-substituted pyridazine with bromoacetyl compounds as shown in Scheme 4. No 2-unsubstituted compounds could be detected in any of these preparations (Quaternization of 3-aminopyridazine with methyl iodide [28] did not proceed at the amino group; and steric hindrance by a 6-substituent will favor quaternization at N-2). The structures of some 3-unsubstituted (and 3-benzamidomethyl and 3-methoxy)imidazo[1,2-*b*]-pyridazines have been established by X-ray analyses [17,44,45]. The proton at position 3 (^{1}H nmr, δ 7.80-8.20) could be replaced under the conditions of the Mannich reaction to give 3-dimethylaminomethyl compounds [46] and by *N*-hydroxymethylamides [46] to give 3-acylaminomethyl compounds [46]. Some 3-unsubstituted imidazo-[1,2-*b*]pyridazines with ethyl glyoxylate gave the ethyl (imidazo[1,2-*b*]pyridazin-3-yl)-2-hydroxyacetate [47], and with ethyl 2-acetoxy-*N*-acetylglycinate gave the ethyl 2-acetamido(imidazo[1,2-*b*]pyridazin-3-yl)acetate [47] Scheme 4.

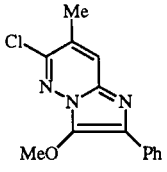
Scheme 3



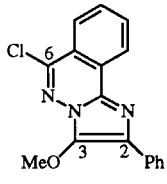
Scheme 4



The preparation of substituted imidazo[1,2-*a*]pyridines **2** [45,48-51] imidazo[1,2-*a*]pyrimidines **3** [48] and imidazo[1,2-*a*]pyrazines **4** [48] for direct comparison with the substituted imidazo[1,2-*b*]pyridazines prepared above; and of the 7-methyl derivative **14** [19] and the 7,8-benzene annelated derivative **15** (**221**) (6-chloro-3-methoxy-2-phenylimidazo[1,2-*a*]phthalazine) [19] of **23** have been described.



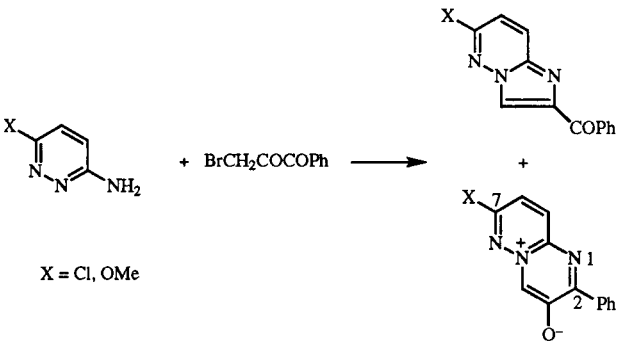
14



15

The reactions of 3-amino-6-chloro(or methoxy)pyridazine with 3-bromo-1-phenylpropane-1,2-dione differed from the reactions with simple α-bromoacetyl compounds discussed above in that it gave two products, the expected 2-benzoyl-6-chloro(or methoxy)imidazo[1,2-*b*]pyridazine [44,47] together with 7-chloro(or 7-methoxy)-2-phenylpyrimido[1,2-*b*]pyridazine-5-ium-3-olate **16** (Scheme 5) [47]. 3-Amino-6-phenylthiopyridazine under similar conditions [47] gave no 2-benzoyl-6-phenylthioimidazo[1,2-*b*]-pyridazine but only 2-phenyl-7-phenylthiopyrimido[1,2-*b*]-pyridazin-5-ium-3-olate [47].

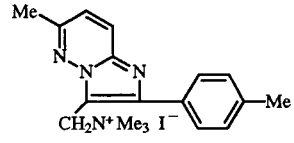
Scheme 5



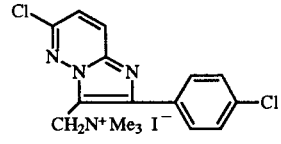
16

Whereas 3-amino-6-chloropyridazine 2-oxide with 3,4-methylenedioxybenzylamine at 145° for 12 hours gave 3-amino-6-(3,4-methylenedioxybenzylamino)pyridazine 2-oxide [52], a similar reaction at 160° for 16 hours also gave a significant quantity of 3-amino-6-(3,4-methylenedioxybenzylamino)pyridazine by removal of the *N*-oxide group [52].

Our attempts to prepare the imidazo[1,2-*b*]pyridazine analogues of zolpidem and alpidem were not successful. 6-Methyl-2-(*p*-tolyl)-3-trimethylammoniomethylimidazo-[1,2-*b*]pyridazine iodide **17**, 6-methyl-3-methylsulphonylmethyl-2-(*p*-tolyl)imidazo[1,2-*b*]pyridazine and 6-chloro-2-(*p*-chlorophenyl)-3-trimethylammoniomethylimidazo-[1,2-*b*]pyridazine iodide **18** could not be converted with potassium cyanide to the corresponding 3-cyanomethyl compounds [47].



17



18

Biological Activity.

Binding to Central and Mitochondrial Benzodiazepine Receptors.

The compounds prepared in this work were tested for their ability, or otherwise, to displace [^{3}H]diazepam from central benzodiazepine receptors (isolated from rat forebrain membrane) in the presence of γ-aminobutyric acid, and some were tested for their ability to displace [^{3}H]diazepam from peripheral-type (mitochondrial) benzodiazepine receptors (isolated from rat kidney membrane) in the absence of γ-aminobutyric acid. Details of the test procedures for the determination of binding have been published for the central benzodiazepine receptors [19] and for the peripheral-type (mitochondrial) benzodiazepine receptors [45,53]. Some of these results are listed, with references [19,44-52,54-66] in Table 1 as IC_{50} values (the concentration required to inhibit standard binding by 50%) (n*M*) or as the percentage inhibition of binding at 1000 n*M* (in parentheses). Formula numbers **19-223** are given for ease of comparison in the text and substituents are defined in Table 1. The initial aim of the work was to establish the structural parameters which influence increased binding ability within series of compounds and then to prepare molecules with the highest selective affinity for the central benzodiazepine receptors or peripheral-type benzodiazepine receptors.

Table 1

Results for the Displacement of [^{3}H]diazepam from the Central Benzodiazepine Receptors and Peripheral-Type Benzodiazepine Receptors by some Substituted Imidazo[1,2-*b*]pyridazines **1**, Imidazo[1,2-*a*]pyridines **2**, Imidazo[1,2-*a*]pyrimidines **3** and Imidazo[l,2-*a*]pyrazines **4**

Assays for displacement from the central benzodiazepine receptors were conducted in the presence of 100 μM γ-aminobutyric acid under the standard assay conditions described in reference [19].

Assays for displacements from the peripheral-type benzodiazepine receptors were conducted in the absence of γ-aminobutyric acid as described in reference [45]. The results are given as IC_{50} values (n*M*) or as percentage inhibitions of control binding at 1000 n*M* (in parentheses).

Compound Number	Substituents X (position 6)	Substituents Y (position 3)	Substituents Z (position 2)	IC_{50} (n*M*) (or % displacement) [a] Central Benzodiazepine Receptors	IC_{50} (n*M*) (or % displacement) [a] Peripheral-Type Benzodiazepine Receptors	[References]
Imidazo[1,2-*b*]pyridazine (**1**)						
19	H	OMe	Ph	3380	-	54
20	Ph	OMe	Ph	>10000	-	55
21	Cl	H	Ph	>3000	(0%)	46,55
22	Cl	OMe	Me	>>1000	-	19
23	Cl	OMe	Ph	775	-	19
24	F	OMe	Ph	320	-	19
25	F	OMe	C_6H_4Me-*p*	17.9	>1000	54,55
26	Br	OMe	Ph	3168	-	19
27	Cl	OEt	Ph	508	-	19
28	Cl	OPr	Ph	380	-	55
29	Cl	OCH_2CH_2OH	Ph	626	-	55
30	Cl	OCH_2CH_2OEt	Ph	164	-	55
31	Cl	OCH_2Ph	Ph	352	-	55
32	Cl	OC_6H_{11}	Ph	>>1000	(35%)	55
33	Cl	OMe	C_6H_4Me-*o*	(8.9%)	-	19
34	Cl	OMe	C_6H_4Me-*m*	1284	-	19
35	Cl	OMe	C_6H_4Me-*p*	148	-	19
36	Cl	OMe	C_6H_3(3,4-OCH_2O)	84	-	56
37	Cl	OMe	C_6H_4OMe-*m*	960	-	19
38	Cl	OMe	C_6H_4OMe-*p*	267	-	19
39	Cl	OMe	C_6H_4Cl-*p*	207	-	19
40	Cl	OMe	C_6H_4F-*p*	462	-	19
41	Cl	OMe	$C_6H_4NH_2$-*m*	609	-	19
42	Cl	OMe	$C_6H_4NH_2$-*p*	403	-	19
43	Cl	OMe	$C_{10}H_7$-α	13000	-	19
44	Cl	OMe	$C_{10}H_7$-β	451	-	19
45	SMe	OMe	Ph	884	-	19
46	SO_2Me	OMe	Ph	>>1000	-	55
47	SEt	OMe	Ph	310	-	50
48	SPr	OMe	Ph	164	-	50
49	SC_6H_{13}	OMe	Ph	653	-	50
50	SC_6H_{11}	OMe	Ph	3399	-	50
51	SPh	OMe	Ph	117	-	50
52	SC_6H_4OMe-*o*	OMe	Ph	28	-	50
53	SC_6H_4OMe-*m*	OMe	Ph	26	-	50
54	SC_6H_4OMe-*m*	OMe	C_6H_3(3,4-OCH_2O)	15	(50%)	55
55	SC_6H_4F-*p*	OMe	Ph	251	-	50
56	$SC_6H_4NMe_2$-*p*	OMe	Ph	97	-	50
57	OMe	OMe	C_6H_4Me-*p*	191	-	57
58	OPh	OMe	Ph	1120	(15%)	55,58
59	OC_6H_4OMe-*o*	OMe	Ph	70	-	59
60	OC_6H_4OEt-*o*	OMe	Ph	50	-	59
61	OC_6H_4OMe-*o*	OMe	C_6H_4Me-*p*	64	-	59
62	OC_6H_4OMe-*o*	OMe	C_6H_4F-*p*	30	-	59
63	OC_6H_4OMe-*o*	OMe	C_6H_3(3,4-OCH_2O)	19	(26%)	55,60
64	OC_6H_4OMe-*o*	OMe	$C_6H_4NO_2$-*m*	18	-	60
65	SCH_2Ph	OMe	Ph	25	-	61
66	$SCH_2C_6H_4$OMe-*o*	OMe	Ph	9	-	59
67	$SCH_2C_6H_4$OMe-*m*	OMe	Ph	11	-	59

(continued)

Table 1 (continued)

Compound Number	Substituents X (position 6)	Substituents Y (position 3)	Z (position 2)	IC_{50} (n*M*) (or % displacement) [a] Central Benzodiazepine Receptors	IC_{50} (n*M*) (or % displacement) [a] Peripheral-Type Benzodiazepine Receptors	[References]
Imidazo[1,2-*b*]pyridazine (1)						
68	$SCH_2C_6H_4OMe$-*m*	OMe	C_6H_4F-*p*	5	>1000	55,59
69	$SCH_2C_6H_4OMe$-*p*	OMe	Ph	55	-	59
70	$SCH_2C_6H_4Cl$-*o*	OMe	C_6H_4Me-*p*	54	-	61
71	$SCH_2C_6H_4NMe_2$-*p*	OMe	C_6H_4Me-*p*	603	-	61
72	$SCH_2C_6H_4OMe$-*m*	OMe	C_6H_4Me-*p*	17	-	59
73	$SCH_2C_6H_4OMe$-*m*	OMe	$C_6H_4NH_2$-*m*	5	-	59
74	$SCH_2C_6H_4OMe$-*m*	OMe	C_5H_4N-β	5	-	59
75	$SCH_2C_6H_4OMe$-*m*	OMe	C_5H_4N-γ	37	-	59
76	OCH_2Ph	OMe	Ph	20	-	62
77	$OCH_2C_6H_4OMe$-*m*	OMe	Ph	6	>1000	55,62
78	$OCH_2C_6H_4OMe$-*m*	OMe	C_6H_4F-*p*	1.5	-	62
79	NHPh	OMe	C_6H_4Me-*p*	>>1000	(48%)	55,63
80	$NHCH_2Ph$	OMe	Ph	9	-	63
81	$NHCH_2CH_2Ph$	OMe	Ph	800	(25%)	55,63
82	$NHCH_2C_6H_4OMe$-*o*	OMe	Ph	1.8	-	63
83	$NHCH_2C_6H_4OMe$-*o*	OMe	C_6H_4Me-*p*	5.5	-	63
84	$NHCH_2C_6H_4OMe$-*o*	OMe	C_6H_3(3,4-CH_2O)	0.3	(40%)	64
85	$NHCH_2C_6H_4OMe$-*o*	OMe	C_6H_4Cl-*p*	16	-	63
86	$NHCH_2C_6H_4OMe$-o	OMe	C_6H_4F-*p*	2.2	-	63
87	$NHCH_2C_6H_4OMe$-*o*	OMe	C_5H_4N-β	3	-	63
88	$NHCH_2C_6H_4OMe$-*m*	OMe	Ph	2.9	>1000	55,63
89	$NHCH_2C_6H_4OMe$-*m*	OMe	C_6H_4Me-*p*	3.2	-	63
90	$NHCH_2C_6H_4OMe$-*m*	OMe	C_6H_3(3,4-OCH_2O)	1.5	(45%)	52,55
91	$NHCH_2C_6H_4OMe$-*m*	OMe	C_6H_4F-*p*	1.5	-	63
92	$NHCH_2C_6H_4OMe$-*m*	OMe	C_5H_4N-β	2.1	-	63
93	$NHCH_2C_6H_4OMe$-*m*	OMe	$C_6H_4NH_2$-*m*	1.8	-	63
94	$NHCH_2C_6H_4OMe$-*m*	OMe	$C_6H_4NH_2$-*p*	1	-	63
95	$NHCH_2C_6H_4OMe$-*m*	OCH_2CH_2OEt	Ph	1	-	52
96	$NHCH_2C_6H_3(OMe)_2$-*m,p*	OMe	C_6H_3(3,4-OCH_2O)	2	(40%)	52,55
97	$NHCH_2C_6H_3(OMe)_2$-*m,p*	OMe	$C_6H_4NH_2$-*p*	3.2	-	52
98	$NHCH_2C_6H_3$(3,4-OCH_2O)	OMe	C_6H_3(3,4-OCH_2O)	1	-	52
99	$SCH_2C_5H_4N$-α	OMe	Ph	4.8	(12%)	64
100	$SCH_2C_5H_4N$-α	OMe	C_6H_3(3,4-OCH_2O)	1.7	(39%)	64
101	$SCH_2C_5H_4N$-β	OMe	Ph	7	-	64
102	$SCH_2C_5H_4N$-β	OMe	C_6H_3(3,4-OCH_2O)	2.1	(82%)	64
103	$SCH_2C_5H_4N$-γ	OMe	Ph	6.1	(21%)	64
104	$SCH_2C_5H_4N$-γ	OMe	C_6H_3(3,4-OCH_2O)	4.7	(24%)	64
105	$NHCH_2C_5H_4N$-β	OMe	Ph	6.9	(40%)	64
106	$NHCH_2C_5H_4N$-β	OMe	C_5H_4N-β	36	-	64
107	Cl	OMe	CH_2Ph	>>10000	-	65
108	Cl	OMe	CH=CHPh	(38%)	(3%)	44,55
109	Cl	OMe	$C_6H_4C_6H_5$-*p*	(9%)	-	55,65
110	OC_6H_4OMe-*o*	OMe	C_6H_{11}	(8%)	-	55
111	SCH_2Ph	OMe	*t*-Bu	(5%)	-	55
112	$SCH_2C_6H_4OMe$-*m*	OMe	CH_2Ph	114	-	55,65
113	$SCH_2C_6H_4OMe$-*m*	OMe	CH_2CH_2Ph	>>1000	-	55,65
114	H	$CH_2NHCOPh$	Ph	214	>1000	51,54
115	Me	H	Ph	(24%)	(0%)	55
116	Cl	H	Ph	>3000	(0%)	45,46
117	Cl	H	C_6H_4Me-*p*	527	-	46
118	Cl	H	C_6H_3(3,4-OCH_2O)	1427	(23%)	46,55
119	Cl	CH_2NHCOH	Ph	847	(65%)	45
120	Cl	$CH_2NHCOMe$	Ph	474	177	45,46
121	Cl	$CH_2NHCOEt$	Ph	198	32	45
122	Cl	$CH_2NHCOPr$	Ph	56	36	45
123	Cl	$CH_2NHCOPh$	Ph	140	114	45,46
124	Cl	$CH_2NHCOPh$	COPh	(40%)	257	44,45
125	Cl	$CH_2NHCOPh$	C_6H_4Me-*p*	18	-	46

(continued)

Table 1 (continued)

Compound Number	Substituents X (position 6)	Substituents Y (position 3)	Substituents Z (position 2)	IC_{50} (n*M*) (or % displacement) [a] Central Benzodiazepine Receptors	IC_{50} (n*M*) (or % displacement) [a] Peripheral-Type Benzodiazepine Receptors	[References]
Imidazo[1,2-*b*]pyridazine (**1**)						
126	Cl	$CH(COOEt)OH$	C_6H_4Me-*p*	24	(91%)	47
127	Cl	$CH_2NHCOMe$	$C_6H_3(3,4\text{-}OCH_2O)$	152	-	46
128	Cl	$CH_2NHCOPh$	$C_6H_3(3,4\text{-}OCH_2O)$	25	-	46
129	Cl	CH_2NMe_2	$C_6H_3(3,4\text{-}OCH_2O)$	2043	(22%)	46,55
130	Cl	H	C_6H_4Cl-*p*	(46%)	(14%)	46,51
131	Cl	CH_2NHCOH	C_6H_4Cl-*p*	(71%)	59	51
132	Cl	$CH_2NHCOMe$	C_6H_4Cl-*p*	92	4	51
133	Cl	$CH_2NHCOEt$	C_6H_4Cl-*p*	40	5	51
134	Cl	$CH_2NHCOPr$	C_6H_4Cl-*p*	11	6	51
135	Cl	$CH_2NHCOPh$	C_6H_4Cl-*p*	26	8	51
136	Cl	H	C_6H_4I-*p*	(41%)	(54%)	51
137	Cl	$CH_2NHCOMc$	C_6H_4I-*p*	235	1.6	51
138	Cl	$CH_2NHCOC_6H_4Cl$-*o*	C_6H_4I-*p*	329	4.1	51
139	Cl	$CH_2NHCOC_6H_4Cl$-*p*	C_6H_4I-*p*	370	3.0	51
140	Cl	H	C_6H_4-*t*-Bu-*p*	(12%)	(4%)	45
141	Cl	$CH_2NHCOMe$	C_6H_4-*t*-Bu-*p*	(39%)	10	45
142	Cl	$CH_2NHCOEt$	C_6H_4-*t*-Bu-*p*	1183	7	45
143	Cl	$CH_2NHCOPr$	C_6H_4-*t*-Bu-*p*	1192	9	45
144	Cl	$CH_2NHCOPh$	C_6H_4-*t*-Bu-*p*	(15%)	6.2	45
145	Cl	$CH_2NHCOC_6H_4F$-*o*	C_6H_4-*t*-Bu-*p*	(18%)	(69%)	45
146	Cl	$CH_2NHCOC_6H_4F$-*m*	C_6H_4-*t*-Bu-*p*	(42%)	(52%)	45
147	Cl	$CH_2NHCOC_6H_4F$-*p*	C_6H_4-*t*-Bu-*p*	(8%)	<1	45
148	Cl	H	$C_6H_4C_6H_5$-*p*	(5%)	(19%)	51
149	Cl	$CH_2NHCOMe$	$C_6H_4C_6H_5$-*p*	(0%)	2.8	51
150	Cl	H	CH=CHPh	(8%)	(26%)	44,45
151	Cl	$CH_2NHCOPh$	CH=CHPh	(17%)	5	44,45
152	Cl	H	$C_6H_4C_6H_{11}$-*p*	(0%)	(3%)	45
153	Cl	$CH_2NHCOPh$	$C_6H_4C_6H_{11}$-*p*	(19%)	23	45
154	Cl	$CH(COOEt)OH$	C_6H_4Me-*p*	24	(91%)	47
155	F	H	C_6H_4Me-*p*	383	(13%)	46,55
156	F	$CH_2NHCOPh$	C_6H_4Me-*p*	8	168	46,55
157	I	$CH_2NHCOPh$	Ph	229	(90%)[b]	51
158	I	H	C_6H_4-*t*-Bu-*p*	(2%)	(8%)	51
159	I	$CH_2NHCOMe$	C_6H_4-*t*-Bu-*p*	(8%)	11	51
160	I	$CH_2NHCOPh$	C_6H_4-*t*-Bu-*p*	(9%)	4.2	51
161	I	$CH(COOEt)OH$	C_6H_4-*t*-Bu-*p*	(10%)	84	47
162	SMe	H	C_6H_4Me-*p*	(35%)	(11%)	46,55
163	SMe	H	$C_6H_3(3,4\text{-}OCH_2O)$	(40%)	(29%)	46,55
164	SMe	$CH_2NHCOPh$	C_6H_4Me-*p*	7	(72%)	46,55
165	SMe	$CH_2NHCOPh$	$C_6H_3(3,4\text{-}OCH_2O)$	2	(~60%)	46,55
166	SPh	H	Ph	(43%)	(31%)	46,55
167	SPh	$CH_2NHCOMe$	Ph	24	(48%)	46,55
168	SPh	$CH_2NHCOPh$	Ph	9	(65%)	46,55
169	SC_6H_4OMe-*o*	H	Ph	(55%)	-	50
170	SC_6H_4OMe-*o*	$CH_2NHCOPh$	Ph	(23%)	71	50,55
171	OMe	H	C_6H_4Me-*p*	1704	(21%)	45,46
172	OMe	$CH_2NHCOPh$	C_6H_4Me-*p*	23	(71%)	45,46
173	OMe	H	$C_6H_3(3,4\text{-}OCH_2O)$	(58%)	-	57
174	OMe	$CH_2NHCOPh$	$C_6H_3(3,4\text{-}OCH_2O)$	7	-	57
175	OMe	H	C_6H_4-*t*-Bu-*p*	(14%)	(22%)	45
176	OMe	$CH_2NHCOPh$	C_6H_4-*t*-Bu-*p*	(52%)	32	57
177	OEt	H	$C_6H_3(3,4\text{-}OCH_2O)$	(54%)	-	57
178	OEt	$CH_2NHCOPh$	$C_6H_3(3,4\text{-}OCH_2O)$	25	-	57
179	SCH_2Ph	H	Ph	428	-	46
180	SCH_2Ph	$CH_2NHCOMe$	Ph	55	(51%)	46,55
181	SCH_2Ph	$CH_2NHCOPh$	Ph	445	-	46
182	Me	CH_2OMe	C_6H_4Me-*p*	139	(17%)	47
183	Me	CH_2SMc	C_6H_4Me-*p*	695	1082	47
184	Me	CH_2SO_2Me	C_6H_4Me-*p*	(15%)	92	47

(continued)

Table 1 (continued)

Compound Number	Substituents X (position 6)	Substituents Y (position 3)	Substituents Z (position 2)	IC_{50} (n*M*) (or % displacement) [a] Central Benzodiazepine Receptors	IC_{50} (n*M*) (or % displacement) [a] Peripheral-Type Benzodiazepine Receptors	[References]
Imidazo[1,2-*a*]pyrimidine (**3**)						
185	Cl	OMe	C_6H_4Me-*p*	>>3000	-	48
186	Cl	H	C_6H_4Me-*p*	(0%)	(45%)	48,55
187	Cl	$CH_2NHCOPh$	C_6H_4Me-*p*	504	(39%)	48,55
Imidazo[1,2-*a*]pyrazine (**4**)						
188	Cl	OMe	C_6H_4Me-*p*	(10%)	-	48
189	Cl	H	C_6H_4Me-*p*	(3%)	(34%)	48,55
190	Cl	$CH_2NHCOPh$	C_6H_4Me-*p*	(6%)	-	48
Imidazo[1,2-*a*]pyridine (**2**)						
191	Cl	OMe	Ph	(61%)	(24%)	49
192	Cl	OMe	C_6H_4Me-*p*	146	(60%)	48,55
193	SPh	OMe	C_6H_4Me-*p*	(11%)	-	48
194	H	H	Ph	(6%)	(17%)	51
195	Cl	H	Ph	(26%)	(64%)	49,51
196	Cl	$CH_2NHCOPh$	Ph	47	(65%)	49,51
197	Cl	$CH_2NHCOPh$	COPh	(15%)	(46%)	66
198	Cl	H	C_6H_4Cl-*p*	(42%)	(84%)	51
199	Cl	$CH_2NHCOPh$	C_6H_4Cl-*p*	17	(86%)	51
200	Cl	CH(COOEt)NHCOPh	C_6H_4Cl-*p*	(13%)	13	66
201	Cl	H	C_6H_4-*t*-Bu-*p*	(47%)	134	51
202	Cl	$CH_2NHCOPh$	C_6H_4-*t*-Bu-*p*	(7%)	487	51
203	I	H	Ph	(11%)	(62%)	51
204	I	$CH_2NHCOPh$	Ph	(19%)	(71%)	51
205	SMe	H	C_6H_4Me-*p*	(22%)	(17%)	51
206	SMe	$CH_2NHCOPh$	C_6H_4Me-*p*	27	-	48,55
207	SPh	H	Ph	(2%)	(26%)	48,55
208	SPh	$CH_2NHCOMe$	Ph	455	(46%)	48,55
209	SPh	$CH_2NHCOPh$	Ph	(37%)	(42%)	48,55
210	OMe	H	C_6H_4Me-*p*	(9%)	(41%)	48,55
211	OMe	$CH_2NHCOPh$	C_6H_4Me-*p*	25	(42%)	48,55
212 (**6**) (alpidem)	Cl	CH_2CONPr_2	C_6H_4Cl-*p*	5.3	4.8	66
213 (**7**) (zolpidem)	Me	CH_2CONMe_2	C_6H_4Me-*p*	31	387	66
Trisubstituted Imidazo[1,2-*a*]pyridines						
214	7-Cl-3-$CH_2NHCOPh$-2-Ph			(58%)	(48%)	49,55
215	7-Cl-3-$CH_2NHCOPh$-2-C_6H_4Me-*p*			(68%)	(58%)	49,55
216	8-Cl-3-$CH_2NHCOPh$-2-Ph			(12%)	(17%)	49,55
217	8-Cl-3-$CH_2NHCOPh$-2-C_6H_4Me-*p*			(16%)	131	49,55
218	8-OMe-3-$CH_2NHCOPh$-2-Ph			(32%)	(13%)	49,55
Tetrasubstituted Imidazo[1,2-*b*]pyridazines						
219	6=0-5-Me-3-OMe-2-C_6H_4Me-*p*			26	(18%)	55,57
220 (**14**)	7-Me-6-Cl-3-OMe-2-Ph			(24%)	-	19
Imidazo[2,1-*a*]phthalazine						
221 (**15**)	6-Cl-3-OMe-2-Ph			>10,000	-	19
Pyrimido[1,2-*b*]pyridazin-5-ium-3-olate (**16**)						
222	7-Cl-2-Ph			(5%)	(14%)	47
Reference Compound						
223	Diazepam			4.3	73	-

[a] at 1000 n*M*; [b] 50% at 100 n*M*.

An examination of the data in Table 1 for binding to the central benzodiazepine receptors by the 3-methoxy compounds revealed that whereas compound **23** (6-chloro-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine) displaced [^{3}H]diazepam from the central benzodiazepine receptors (IC_{50} 775 n*M*, our initial discovery of binding in this series of compounds), compounds **19** and **21** (which lacked the 6-chloro- and 3-methoxy-substituents respectively) and compound **22** (which lacked an aromatic substituent at the 2-position) were much less effective in displacing [^{3}H]diazepam from the central benzodiazepine receptors. Also compound **20** (with a 6-phenyl substituent) was much less efficient in binding than was the 6-chloro analogue **23.** The 7-methyl derivative **14** (**220**) [19] and the 7,8-benzene annelated derivative **15** (**221**) [19] were markedly less active than compound **23**, probably for reasons of steric hindrance at the receptor site.

Of the simple 6-halogeno compounds **23**, **24** and **26** examined, the 6-fluoro compound **24** bound most strongly; but exploratory work, for reasons of ease of syntheses, was effected with 6-chloro compounds. Amongst the various 3-alkoxy compounds, compound **30** (IC_{50} 164 n*M*) with the 3-(2-ethoxyethoxy) group bound most strongly and the 3-cyclohexyloxy compound **32** (IC_{50} >> 1000 n*M*) bound least strongly. Some 6-chloro-2-(substituted phenyl)-compounds also exhibited enhanced binding ability compared to their 2-phenyl analogue. This was most noticeable in the 2-*p*-tolyl-, 2-(3,4-methylenedioxyphenyl) and 2-*p*-chlorophenyl-compounds **35**, **36** and **39** respectively. The 2-β-naphthyl compound **44** was markedly more active than its α-isomer **43**.

Amongst the 6-alkylthio compounds, the 6-methylthio compound **45** had approximately the same affinity as its 6-chloro analogue **23**, and of the compounds examined the 6-propylthio compound **48** bound most strongly. When the 6-methylthio compound was oxidized, the resulting 6-methylsulphonyl compound **46** did not bind significantly.

Consideration of the 6-arylthio(or 6-aryloxy) compounds revealed that methoxy groups as in 6-(*o*- or *m*-methoxyphenylthio)- or 6-(*o*-methoxyphenoxy) substituents as in compounds **52**, **53** and **59** significantly enhanced binding; and further enhancement was observed when a 2-(3,4-methylenedioxyphenyl) group was present as in compound **63**. The beneficial effects of methoxy substituents in the 6-(*o*- or *m*-methoxybenzylthio) and 6-(*m*-methoxybenzyloxy) compounds **66**, **67** and **77** relative to the 6-benzylthio- and 6-benzyloxy compounds **65** and **76** respectively were clearly revealed. Further enhancement of activity is apparent in compounds **73** with a 2-(*m*-aminophenyl) group, **74** with a 2-(pyridin-3-yl) group, and **78** with a 2-(*p*-fluorophenyl) group. Remarkable differences in binding affinity were observed in the 6-anilino and 6-aralkylaminoimidazo[1,2-*b*]pyridazines: the 6-benzylamino compound **80** had high activity whereas the 6-anilino **79** and 6-(2-phenethylamino) compounds **81** were relatively inactive. (The differences in affinity between compounds **80** and **79** was greater than between the 6-benzyloxy- and 6-phenoxy compounds **76** and **58** respectively. A large number of 6-(methoxybenzylamino)-3-methoxy-2-arylimidazo[1,2-*b*]pyridazines **82-97** containing beneficial 2-aryl groups discussed above, with high affinity for the central benzodiazepine receptors have been prepared and are listed in Table 1. The most active compound was 2-(3,4-methylenedioxyphenyl)-3-methoxy-6-(*o*-methoxybenzylamino)imidazo[1,2-*b*]pyridazine **84** (IC_{50} 0.3 n*M*). This should be compared with diazepam **223** (IC_{50} 4.3 n*M*).

Similarly a significant number of 3-methoxy-6-(α- β- and γ-picolylthio- and β-picolylamino)-2-(phenyl and 3,4-methylenedioxyphenyl)imidazo[1,2-*b*]pyridazines **99-105** have been shown to have IC_{50} values in the range 1.7-7.0 n*M* for the displacement of [^{3}H]diazepam from the central benzodiazepine receptors.

A large number of 3-methoxy-2,6-disubstituted imidazo[1,2-*b*]pyridazines have been prepared [65] which contain 2-(benzyl, phenethyl, styryl, 4-biphenylyl, *t*-butyl, or cyclohexyl) groups and the test data of a selection **107-113** listed in Table 1 show that, in comparison to their 2-phenyl analogues **23**, **59**, **65** and **67**, all showed low affinity for the central benzodiazepine receptors.

Tests to measure the displacement of [^{3}H]diazepam from the peripheral-type benzodiazepine receptors by some of the 3-methoxy compounds revealed displacements of <50% at 1000 n*M* for all except compound **102** (82% at 1000 n*M*). Thus the 3-methoxy compounds are selective for the central benzodiazepine receptors.

The imidazo[1,2-*b*]pyridazin-6(5*H*)-one **219** had a much higher affinity for the central benzodiazepine receptors than its 6-methoxy isomer **57**; and 7-chloro-2-phenylpyrimido[1,2-*b*]pyridazin-5-ium-3-olate **222** was practically devoid of affinity for the central benzodiazepine receptors or peripheral-type benzodiazepine receptors.

Examination of a range of 3-(acylaminomethyl, dimethylaminomethyl and unsubstituted)imidazo[1,2-*b*]-pyridazines revealed a quite different situation. Whereas the 3-(dimethylaminomethyl and unsubstituted) compounds showed little affinity for the central benzodiazepine receptors or peripheral-type benzodiazepine receptors, some 3-acylaminomethyl compounds were highly selective for the peripheral-type benzodiazepine receptors (but others bound strongly to the central benzodiazepine receptors).

Amongst the 6-chloroimidazo[1,2-*b*]pyridazines, compound **116** showed little affinity for the central benzodiazepine receptors or peripheral-type benzodiazepine

receptors but the 3-acylaminomethyl derivatives **119-123** showed markedly increased affinity for both receptors. The 2-(*p*-chlorophenyl) compounds **131-135** exhibited an even higher affinity (than their 2-phenyl analogues) for both receptors but with a preferential affinity for the peripheral-type benzodiazepine receptors. Further enhancement in selectivity for the peripheral-type benzodiazepine receptors was found for the 2-(*p*-iodophenyl) compounds (*e.g.* **137**) and the 2-(4-*t*-butylphenyl) compounds (*e.g.* **141**). The 2-(4-biphenylyl, styryl and 4-cyclohexylphenyl) groups in such systems (*e.g.* **149**, **151** and **153**) also produced preferential binding to the peripheral-type benzodiazepine receptors.

The 6-fluoro compound **156** showed greater selectivity and greater affinity for the central benzodiazepine receptors (relative to the peripheral-type benzodiazepine receptors) than the 6-chloro- or 6-iodo-compounds **123** or **157** respectively which exhibited slight preferential affinity for the peripheral-type benzodiazepine receptors. 3-Benzamidomethyl-2-(4-*t*-butylphenyl)-6-iodoimidazo[1,2-*b*]-pyridazine **160** had very strong preferential affinity for the peripheral-type benzodiazepine receptors.

6-(Methylthio and phenylthio) groups as in compounds **164**, **165** and **168** were beneficial for stronger preferential affinity for the central benzodiazepine receptors relative to their 6-chloro analogues **125**, **128** and **123** whereas the 6-(*o*-methoxyphenylthio) compound **170** was a much weaker ligand for the central benzodiazepine receptors. The 6-benzylthio compounds **180**, **181** had lower affinity for the central benzodiazepine receptors than their 6-phenylthio analogues **167**, **168**; this contrasts with the observed affinities of the 3-methoxy compounds **65** and **51**.

The 3-methoxymethyl compound **182** (prepared during attempted syntheses of the imidazo[1,2-*b*]pyridazine analogue of zolpidem) was selective for the central benzodiazepine receptors, and its 3-methylthiomethyl analogue **183** was less selective, but the 3-methylsulphonylmethyl compound **184** exhibited a preferential affinity for the peripheral-type benzodiazepine receptors.

Some comparisons of the biological activity of substituted imidazo[1,2-*b*]pyridazines are possible with data presented in Table 1 for substituted imidazo[1,2-*a*]pyrimidines **3**, imidazo[1,2-*a*]pyrazines **4** and imidazo[1,2-*a*]-pyridines **2**. The imidazo[1,2-*a*]pyrimidines **185-187** and imidazo[1,2-*a*]pyrazines **188-190** were much weaker ligands for binding to central benzodiazepine receptors than their imidazo[1,2-*b*]pyridazine analogues **35**, **117** and **125** respectively and will not be discussed further.

Some substituted imidazo[1,2-*b*]pyridines, however, had strong affinity for the central benzodiazepine receptors. Whereas the 6-chloro-3-methoxy compounds **191** and **192** had approximately the same affinity for the central benzodiazepine receptors as compounds **23** and **35**, respectively; the 3-benzamidomethyl-6-chloro compound **196** had *ca* 3 times the affinity for the central benzodiazepine receptors as compound **123** but compound **196** also bound much less strongly to the peripheral-type benzodiazepine receptors. This general pattern was also shown by compounds **199** and **202** relative to compounds **135** and **144**, respectively, but the 6-methoxy compounds **211** and **172** had comparable affinities as ligands to both central benzodiazepine receptors and peripheral-type benzodiazepine receptors.

A comparison of test data for the isomeric imidazo[1,2-*a*]-pyridines revealed that the 6-chloro compound **196** had significantly higher affinity for the central benzodiazepine receptors than its 7- or 8-chloro isomers **214**, **216**; and that 3-benzamidomethyl-8-chloro-2-*p*-tolylimidazo[1,2-*a*]-pyridine **217**, unlike its isomers, had relatively high affinity (IC_{50} 131 n*M*) for the peripheral-type benzodiazepine receptors.

In summary, the limited results for the 6-substituted imidazo[1,2-*a*]pyridines and those of the imidazo[1,2-*b*]-pyridazines indicate that the latter are more selective ligands for the peripheral-type benzodiazepine receptors than the former.

As several tumors (including gliomas) and certain neurodegenerative disorders (such as Huntingtons disease) have displayed enhanced peripheral-type benzodiazepine receptors densities, some of our compounds with high selective affinity for the peripheral-type benzodiazepine receptors are currently being examined [67] as potential radiopharmaceuticals for imaging these disease states by Positron Emission Tomography or Single Photon Emission Computed Tomography.

γ-Aminobutyric Acid Shift *In Vitro*.

It is well known that receptor affinity for central benzodiazepine receptors agonists but not antagonists is increased in the presence of γ-aminobutyric acid [68-70]. We measured the inhibition of [^{3}H]flumazinil binding *in vitro* in the presence (100 m*M*) and absence of γ-aminobutyric acid for some compounds and the results are set out in Table 2 together with those for the reference compounds.

Four of the imidazopyridazines gave γ-aminobutyric acid shifts of the order observed for the agonists diazepam and oxazepam, whereas four others gave smaller γ-aminobutyric acid shifts like CL 218872 which is known to be a partial agonist [23, 24].

Test Results *In Vivo*.

Some of our compounds have been subjected to biological testing *in vivo*. F. Hoffmann La Roche & Co., Basle, in tests for the prevention of pentylenetetrazole-induced convulsions in mice and rats obtained the following

Table 2

Inhibition of [^{3}H]flumazenil binding: γ-Aminobutyric Acid shift *in vitro*

Compound	IC_{50} (n*M*) Control (No γ-Amino-butyric Acid)	IC_{50} (n*M*) Test with γ-Amino-butyric Acid (100 μ*M*)	Ratio
Agonists			
Diazepam (**233**) [a]	8.5	3.7	2.3 [b]
Oxazepam [a]	46.8	19.7	2.4 [b]
CL 218872 (**5**) [b]	230	140	1.6 [b]
Antagonists			
Ro 15-1788			1.0 [b]
Propyl β-carboline-3-carboxylate	3.4	3.4	0.99 [b]
Methyl β-carboline-3-carboxylate	3.8	5.2	0.73 [b]
Test Compounds			
68	42	17	2.5 [b]
156	42	19	2.2 [b]
88	6.7	3.2	2.1 [b]
25	89	44	2.0 [b]
219	58	35.9	1.62 [c]
167	23	15	1.56 [d]
168	19	12	1.54 [d]
114	919	711	1.29 [d]

[a] Reference [68]; [b] Reference [71]; [c] Reference [57]; [d] Reference [55].

results (unpublished) (ED_{50} mg/kg unless stated otherwise): **89** (mice, 17.9; rats 16.9); **93** (12.8, 11.1); **94** (7.1; 5/8 rats protected at 100 mg/kg); **92** (7/8 mice protected at 100 mg/kg; 3.2) respectively. Some species specificity was observed. Compound **88** was orally active in the Geller-Seifert type conflict test in rats at a does of 0.5 mg/kg but was inactive in mice at a dose of 30 mg/kg.

Measurements of the binding of [^{3}H]flumazinil to mouse brain *in vivo* have been carried out on compounds **89**, **93**, **94** and, like the results for flumazinil and clonazepam, there was no correlation between sedation and displacement under the test conditions.

Structure-Affinity Relationship Studies and Molecular Modeling.

The major structural requirements for imidazo[1,2-*b*]-pyridazines with high binding affinities for the central benzodiazepine receptors have been proposed from structure-activity analyses and molecular modeling [57,72].

These studies show generally that the nitrogen at position 1 and an oxygen present in the 3-substituent are required as hydrogen-binding sites, and that the 2-phenyl ring (together with substituent) is required as a lipophilic site(s) which participate(s) in the interaction with the receptor. The binding sites identified for these compounds are consistent with known benzodiazepine receptor-ligand interaction models. It has also been established for the most active compounds that the aromatic moiety at the 6- or 3-position and the heteroatom present in the 6-substituent represent additional binding sites.

The data given in Table 1 for some imidazo[1,2-*b*]-pyridazines with large substituents such as a 2-(4-*t*-butylphenyl, 4-cyclohexylphenyl and biphenyl-4-yl) groups which have low affinity for the central benzodiazepine receptors, but high affinity for the peripheral-type benzodiazepine receptors, indicates that steric effects are more restrictive in the interaction of these ligands with the central benzodiazepine receptors rather than with peripheral-type benzodiazepine receptors.

Acknowledgments.

I wish to acknowledge the specialized and significant contributions by my co-workers: Dr. L. Davies for the binding data; Dr. P. Harrison, Mr. S. Ireland, Dr. C. Khoo-Beattie, Dr. M. Ngu-Schwemlein, Dr. M. Schmitt and Dr. J. Zhang for the chemistry; and Drs. P. Mátyus and M. Wong for the computational chemistry and molecular modeling.

REFERENCES AND NOTES

[1] G. Heinisch and H. Frank, Progress in Medicinal Chemistry, Vol **27**, G. P. Ellis and G. B. West, eds, Elsevier, Amsterdam, 1990, p 1.

[2] G. Heinisch and H. Kopelant-Frank, Progress in Medicinal Chemistry, Vol **29**, G.P. Ellis and D. K. Luscombe, eds, Elsevier, Amsterdam, 1991, p 141.

[3] S. Moreau, P. Coudert, C. Rubat, D. Gardette, D. Valee-Goyet, J. Couquelet, P. Bastide and P. Tronche, *J. Med. Chem.*, **37**, 2153 (1994).

[4] S. Moreau, P. Coudert, C. Rubat, E. Albuisson and J. Couquelet, *Arzneim.-Forsch./Drug Res.*, **46**, 800 (1996).

[5] Y. Nomoto, H. Takai, T. Ohno, K. Nagashima, K. Yao, K. Yamada, K. Kubo, M. Ichimura, A. Mihara and H. Kase, *J. Med. Chem.*, **39**, 297 (1996).

[6] J. E. Leet, D. R. Schroeder, J. Golik, J. A. Matson, T. W. Doyle, K. S. Lam, S. E. Hill, M. S. Lee, J. L. Whitney and B. S. Krishnan, *J. Antibiot.*, **49**, 299 (1996).

[7] A. Melikian, G. Schlewer, J.-P. Chambon and C. G. Wermuth, *J. Med. Chem.*, **35**, 4092 (1992).

[8] P. Mátyus, J. Kosáry, E. Kasztreiner, N. Makk, E. Diesler, K. Czakó, G. Rablockzky, L. Jaszlits, E. Horváth, Z. Tömösközi, G. Csch, E. Horváth and P. Arányi, *Eur. J. Med. Chem.*, **27**, 107 (1992).

[9] C. V. Denyer, M. T. Reddy, D. C. Jenkins, E. Rapson and S. D. M. Watts, *Arch. Pharm. (Weinheim, Ger.)*, **327**, 95 (1994).

[10] E. Luraschi, F. Arena, A. Sacchi, S. Laneri, E. Abignente, M. D'Amico, L. Berrino and F. Rossi, *Farmaco*, **50**, 349 (1995); *Chem. Abstr.*, **123**, 228090 (1995).

[11] T. F. Walsh, K. J. Fitch, M. MacCoss, R. S. L. Chang, S. D. Kivlighn, V. J. Lotti, P. K. S. Siegl, A. A. Patchett and W. J. Greenlee, *Bioorg. Med. Chem. Letters*, **4**, 219 (1994).

[12] Y. Nitta, F. Yoneda and T. Otaka, Japan Patent 22, 263 (1965); *Chem. Abstr.*, **64**, 3566 (1966).

[13] Y. Nitta, F. Yoneda and T. Otaka, Japan Patent 22, 264 (1965); *Chem. Abstr.*, **64**, 3566 (1966).

[14] Y. Nitta, F. Yoneda and T. Otaka, Japan Patent 22, 265 (1965); *Chem. Abstr.*, **64**, 3566 (1966).

[15] Y. Nitta, F. Yoneda and T. Otaka, Japan Patent 22, 267 (1965); *Chem. Abstr.*, **64**, 3567 (1966).

[16] C. Pfizer and Co., Inc. British Patent 1,135,893 (1968); *Chem. Abstr.*, **70**, 57870 (1969).

[17] G. B. Barlin, I. L. Brown, L. Golič and V. Kaučič, *Aust. J. Chem.*, **35**, 423 (1982).

[18] G. B. Barlin, D. J. Brown, Z. Kadunc, A. Petrič, B. Stanovnik and M. Tišler, *Aust. J. Chem.*, **36**, 1215 (1983).

[19] G. B. Barlin, L. P. Davies and M. M. L. Ngu, *Aust. J. Chem.* **41**, 1149 (1988).

[20] J. D. Albright, D. B. Moran, W. B. Wright, Jr., J. B. Collins, B. Beer, A. S. Lippa and E. N. Greenblatt, *J. Med. Chem.*, **24**, 592 (1981).

[21] A. S. Lippa, J. Coupet, E. N. Greenblatt, C. A. Klepner and B. Beer, *Pharmacol. Biochem. Behav.*, **11**, 99 (1970).

[22] K. Biziere, J. J. Bourguignon, J. P. Chambon, M. Heaulme, A. Perio, S. Tebib and C. G. Wermuth, *Br. J. Pharmacol.*, **90**, 183 (1987).

[23] K. A. Wafford, P. J. Whiting and J. A. Kemp, *Mol. Pharmacol.*, **43**, 240 (1993); *Chem. Abstr.*, **118**, 184110 (1993).

[24] K. L. Hadingham, K. A. Wafford, S. A. Thompson, K. J. Palmer and P. J. Whiting, *Eur. J. Pharmacol., Mol. Pharmacol. Sect.*, **291**, 301 (1995); *Chem. Abstr.*, **124**, 77084 (1996).

[25] W. Martindale, The Extra Pharmacopocia 29th Ed., J. E. F. Reynolds, ed, The Pharmaceutical Press, London, 1989, pp 712, 1630.

[26] C. Faure-Halley, D. Graham, S. Arbilla and S. Z. Langer, *Eur. J. Pharmacol. Mol. Pharmacol. Sect.*, **246**, 283 (1993).

[27] A. Albert, R. Goldacre and J. Phillips, *J. Chem. Soc.*, 2240 (1948).

[28] G. B. Barlin, *J. Chem. Soc., Perkin Trans. I*, 1424 (1976).

[29] G. B. Barlin and J. A. Benbow, *J. Chem. Soc., Perkin Trans. II*, 1385 (1975).

[30] J. H. M. Hill and J. G. Krause, *J. Org. Chem.*, **29**, 1642 (1964).

[31] G. B. Barlin and W. V. Brown, *J. Chem. Soc. (B)*, 648 (1967).

[32] G. B. Barlin and W. V. Brown, *J. Chem. Soc. (B)*, 1435 (1968).

[33] G. B. Barlin and A. C. Young, *J. Chem. Soc. (B)*, 1675 (1971).

[34] G. B. Barlin and J. A. Benbow, *J. Chem. Soc., Perkin Trans. II*, 790 (1974).

[35] G. B. Barlin and J. A. Benbow, *J. Chem. Soc., Perkin Trans. II*, 298 (1975).

[36] G. Coppens, F. Declerk, C. Gillet and J. Nasielski, *Bull. Soc. Chim. Belg.*, **70**, 480 (1961).

[37] N. B. Chapman and D. Q. Russell-Hill, *J. Chem. Soc.*, 1563 (1956).

[38] N. B. Chapman and C. W. Rees, *J. Chem. Soc.*, 1190 (1954).

[39] G. B. Barlin, *Aust. J. Chem.*, **39**, 1803 (1986).

[40] G. B. Barlin and S. J. Ireland, *Aust. J. Chem.*, **40**, 1491 (1987).

[41] R. H. Mizzoni and P. E. Spoerri, *J. Am. Chem. Soc.*, **73**, 1873 (1951).

[42] E. A. Steck, R. P. Brundage and L. T. Fletcher, *J. Am. Chem. Soc.*, **76**, 3225 (1954).

[43] T. Itai and T. Nakashima, *Chem. Pharm. Bull.*, **10**, 936 (1962).

[44] G. B. Barlin, L. P. Davies, P. W. Harrison, N. W. Jacobsen and A. C. Willis, *Aust. J. Chem.*, **47**, 1989 (1994).

[45] G. B. Barlin, L. P. Davies, P. W. Harrison, S. J. Ireland and A. C. Willis, *Aust. J. Chem.*, **49**, 451 (1996).

[46] G. B. Barlin, L. P. Davies, S. J. Ireland, M. M. L. Ngu and J. Zhang, *Aust. J. Chem.*, **45**, 731 (1992).

[47] M. Schmitt, J.-J. Bourguignon, G. B. Barlin and L. P. Davies, *Aust. J. Chem.*, **50**, 779 (1997).

[48] G. B. Barlin, L. P. Davies, S. J. Ireland, M. M. L. Ngu and J. Zhang, *Aust. J. Chem.*, **45**, 877 (1992).

[49] G. B. Barlin, L. P. Davies and P. W. Harrison, *Aust. J. Chem.*, **48**, 1031 (1995).

[50] G. B. Barlin, L. P. Davies and S. J. Ireland, *Aust. J. Chem.*, **49**, 443 (1996).

[51] G. B. Barlin, L. P. Davies and P. W. Harrison, *Aust. J. Chem.*, **50**, 61 (1997).

[52] G. B. Barlin, L. P. Davies, S. J. Ireland and J. Zhang, *Aust. J. Chem*, **46**, 353 (1993).

[53] J. H. Skerritt, S. Chen Chow, G. A. R. Johnston and L. P. Davies, *Neurosci. Letters*, **34**, 63 (1982).

[54] G. B. Barlin, L. P. Davies, B. Glenn, P. W. Harrison and S. J. Ireland, *Aust. J. Chem.*, **47**, 609 (1994).

[55] G. B. Barlin and co-workers, unpublished results.

[56] G .B. Barlin, L. P. Davies, R. A. Davis and P. W. Harrison, *Aust. J. Chem.*, **47**, 2001 (1994).

[57] P. W. Harrison, G. B. Barlin, L. P. Davies, S. J. Ireland, P. Mátyus and M. G. Wong, *Eur. J. Med. Chem.*, **31**, 651 (1996).

[58] G. B. Barlin, L. P. Davies and M. M. L. Ngu, *Aust. J. Chem.*, **41**, 1735 (1988).

[59] G. B. Barlin, L. P. Davies, S. J. Ireland and M. M. L. Ngu, *Aust. J. Chem.*, **42**, 1735 (1989).

[60] G. B. Barlin, L. P. Davies, S. J. Ireland, C. L. Y. Khoo and T. M. T. Nguyen, *Aust. J. Chem.*, **43**, 503 (1990).

[61] G. B. Barlin, L. P. Davies, S. J. Ireland and M. M. L. Ngu, *Aust. J. Chem.*, **42**, 1133 (1989).

[62] G. B. Barlin, L. P. Davies and M. M. L. Ngu, *Aust. J. Chem.*, **42**, 1749 (1989).

[63] G. B. Barlin, L. P. Davies and M. M. L. Ngu, *Aust. J. Chem.*, **42**, 1759 (1989).

[64] G. B. Barlin, L. P. Davies, S. J. Ireland and M. M. L. Ngu-Schwemlein, *Aust. J. Chem.*, **50**, 91 (1997).

[65] G. B. Barlin, L. P. Davies, S. J. Ireland and J. Zhang, *Aust. J. Chem.*, **45**, 1281 (1992).

[66] M. Schmitt, J.-J. Bourguignon, G. B. Barlin and L. P. Davies, *Aust. J. Chem.*, **50**, 719 (1997).

[67] A. Katsifis, L. Naidovski, K. Mardon, F. Mattner, G. Barlin, L. Davies and M. Kassiou, *J. Labelled Comp. Radiopharm.*, **40**, 623 (1997)

[68] J. H. Skerritt, L. P. Davies, S. Chen Chow and G. A. R. Johnston, *Neurosci. Letters*, **32**, 169 (1982).

[69] P. Skolnick, M. M. Schweri, E. F. Williams, V. Y. Moncada and S. M. Paul, *Eur. J. Pharmacol.*, **78**, 133 (1982).

[70] H. Möhler and J. G. Richards, *Nature (London)*, **294**, 763 (1981).

[71] L. P. Davies, G. B. Barlin, S. J. Ireland and M. M. L. Ngu, *Biochemical Pharmacol.*, **44**, 1555 (1992).

[72] P. Mátyus, G. B. Barlin, P. W. Harrison, M. G. Wong and L. P. Davies, *Aust. J. Chem.*, **49**, 435 (1996).