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SUBSTITUTED IMIDAZO[1,2-*b*]PYRIDAZINES

NEW COMPOUNDS WITH ACTIVITY AT CENTRAL AND PERIPHERAL BENZODIAZEPINE RECEPTORS

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Abstract—A large range of substituted imidazo[1,2-*b*]pyridazines have been synthesized, and a number of potent ligands at central benzodiazepine (Bz) receptors on rat brain membranes have been identified in initial binding screens using [^{3}H]diazepam. For those tested more extensively, binding studies conducted in the presence and absence of γ-aminobutyric acid suggest that they were full receptor agonists. Some preliminary evidence was found suggesting some species selectivity, i.e. several of the compounds were more active in *in vivo* tests in rats than in mice. The agonist activity of these 2-phenyl (and substituted phenyl) imidazo[1,2-*b*]pyridazines is consistent with the model of Bz receptor ligands as proposed by Fryer [Raven Press, 1983, pp. 7–20]. Several compounds were identified which had more selective activity at peripheral-type (mitochondrial) Bz binding sites. Thus, substituted imidazo[1,2-*b*]pyridazines represent yet another class of low molecular mass compounds which have activity at Bz receptor sites.

The benzodiazepine (Bz‡) class of drugs are used clinically for their anxiolytic, hypnotic, muscle-relaxant and anti-convulsant actions. They act allosterically to influence central γ-aminobutyric acid (GABA)-mediated neurotransmission, rather than directly at neurotransmitter receptors *per se* [1]. Thus, because their effect is limited to modulating the activity of an endogenous transmitter, they have quite low acute toxicity. Nevertheless, there is considerable clinical concern about undesirable side-effects such as sedation, amnesia and, in particular, the ability to induce both physical and psychological dependence [2]. Another problem, particularly with respect to the use of Bz as anticonvulsants, is the quite rapid development of tolerance [3, 4].

New molecules which have the same low acute toxicity but with better pharmacological selectivity (e.g. anxiolytics without sedative action) and a better separation between wanted and unwanted effects may arise from research on Bz-like molecules [5]. Certainly there is significant promise that partial agonists at central Bz receptors may have a reduced potential to cause sedation and dependence [6].

The following work arose from our interest in the possible interaction of purines (e.g. adenosine, methylxanthines) and related nitrogen heterocycles with CNS Bz receptors [7, 8]. A random screen of a number of synthetic nitrogen heterobicycles (including purine compounds) revealed an imidazo[1,2-*b*]pyridazine with good activity in displacing [^{3}H]diazepam bound to rat brain plasma membranes. Further syntheses and structure–activity studies have led to the identification of a number of novel compounds active at central Bz receptors in *in vitro* binding assays and a subset of these imidazo[1,2-*b*]pyridazines with some selectivity for peripheral-type Bz binding sites.

Descriptions of chemical syntheses of a number of imidazopyridazines and results of preliminary screening versus [^{3}H]diazepam binding to washed rat brain plasma membranes (in the presence of 100 μM GABA) have been published [9–16].

MATERIALS AND METHODS

Bz receptor binding in vitro. The binding of [^{3}H]diazepam and [^{3}H]flumazenil was performed essentially as described previously [17, 18]. Diazepam, an agonist for central Bz receptors, can also bind with high affinity to peripheral-type Bz sites while flumazenil (Ro 15-1788) is an antagonist Bz for central receptors.

P2 pellets, prepared from rat (Sprague–Dawley) forebrain homogenates in 0.32 M sucrose by differential centrifugation, were osmotically lysed by suspension in ice-cold distilled water for 20 min and synaptic plasma membranes isolated by further centrifugation. Membranes were washed four to six times for normal screening assays and eight times for *in vitro* GABA shift assays by centrifugation and resuspension in 50 mM Tris-citrate buffer, pH 7.4 (to remove endogenous GABA), and then stored deep-frozen. On the day of use, preparations were washed once in ice-cold distilled water before final

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‡ Abbreviations: Bz, benzodiazepine; GABA, γ-aminobutyric acid; IC_{50}, concentration of compound causing 50% inhibition of binding under standard conditions; PAL, photoaffinity labell(ing/ed).

resuspension in 50 mM Tris–HCl buffer, pH 7.25 (at 0–4°). Test chemicals, dissolved in dimethylsulphoxide, were incubated with the membrane preparation (approx. 0.6 mg protein), 100 μM GABA and [^{3}H]diazepam (0.70 ± 0.05 nM) in a final assay volume of 2 mL of 50 mM Tris-chloride buffer. After 30 min incubation on ice, membranes were collected by filtration on Whatman GF/B filters (2.5 cm dia.) and washed with 3 by 4 mL aliquots of ice-cold buffer. In later studies using a Brandel automated filtration apparatus, assay volumes were reduced to 1 mL and the amount of brain membrane was also reduced. Bound radioactivity was determined by conventional liquid scintillation counting techniques. All samples and controls contained the same amount of dimethylsulphoxide (not more than 10 μL per 2 mL assay). Blanks contained 10 μM unlabelled diazepam.

For *in vitro* GABA shift experiments, assays were conducted using well-washed membranes, both in the presence and absence of 100 μM GABA.

Membranes from rat kidneys were prepared as described previously [19]. Briefly, freshly dissected rat kidneys were rinsed, chopped with scissors and homogenized in 0.32 M sucrose using an Ultra-Turrax homogenizer.

Photoaffinity labelling of rat brain membranes was performed as described previously [20].

Bz binding in vivo. The procedure used for the *in vivo* binding of the Bz antagonist [^{3}H]flumazenil (Ro 15-1788) to whole brains of intact mice was similar to that described previously [21]. Male and female NMRI mice (4–5 per treatment group) were given test compounds by oral gavage (20 mg/kg) and were killed 60 min later by cervical dislocation and decapitation.* Ten minutes before being killed, animals were given a subcutaneous injection of [^{3}H]-flumazenil in normal saline; injection volume was 0.1 mL/10 g body wt, of a 10 μCi/mL solution. Two other groups of animals served as positive controls and received clonazepam or flumazenil (10 mg/kg) 50 min before injection of [^{3}H]flumazenil and 60 min before killing. Brain tissue was processed in two ways; the first method utilized the method of Miller *et al.* [22]. One half of the forebrain was added to 1 mL of Packard Soluene-350 tissue solubilizer in scintillation vials and digested at 35–40° overnight before the addition of toluene/Triton X-100 scintillator and counting to constant background (to allow chemiluminescence to subside). The second procedure essentially utilized the method described by Koe *et al.* [23]. The remainder of the brain was rapidly homogenized in 50 vol. of ice-cold 50 mM Tris-chloride buffer, pH 7.25 and two 1 mL aliquots were added directly to scintillation vials and counted in Optiphase scintillation fluid (total counts in the CNS, i.e. bound and unbound). Another two aliquots were filtered under vacuum onto Whatman GF/B glass-fibre discs (2.5 cm dia.) and washed with 2 × 4 mL aliquots of ice-cold buffer (total bound radioactivity). Another two aliquots were incubated with an excess of unlabelled diazepam (10 μM final concentration) for 25 min prior to filtration and washing (non-specific binding).

Materials. [^{3}H]Diazepam (86.6 Ci/mmol) and [^{3}H]flumazenil (83.2 Ci/mmol) were supplied by Amersham and New England Nuclear, respectively. Diazepam, flumazenil and clonazepam were gifts from Hoffmann-LaRoche (Switzerland). CL218,872 was supplied by Pharmuka Labs (France).

* All procedures involving animals were conducted according to the current "Australian Code of Practice for the Care and Use of Animals for Experimental Purposes".

RESULTS

Initial screening vs [^{3}H]diazepam binding

Arising from the identification of an active imidazo[1,2-*b*]-pyridazine in the initial random screen, structure–activity considerations and further synthesis led to a number of compounds with potent activity in diazepam binding assays *in vitro* using rat forebrain membranes (Table 1 and Fig. 1).

Selectivity for central vs peripheral-type binding sites

A more limited screen of imidazo[1,2-*b*]pyridazines vs [^{3}H]diazepam binding to membranes prepared from rat kidneys (which have a high density of peripheral-type Bz binding sites, located predominantly on outer mitochondrial membranes) revealed that whilst most compounds were selective for central-specific Bz sites, several compounds of those tested in this work showed a significantly higher affinity for peripheral-type sites (Table 2); structures of these compounds are given in Tables 1 and 2.

GABA shift in vitro

It is well known that the receptor affinity of Bz agonists but not antagonists is increased in the presence of GABA [18, 24, 25]. Of four imidazopyridazine compounds tested to date, all showed a GABA shift equivalent to that seen with diazepam and oxazepam (Table 3); CL218,872, a partial agonist, produced a smaller GABA shift.

Interaction with photoaffinity-labelled (PAL) Bz receptors

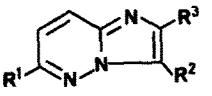
When rat brain synaptic membranes are exposed to UV light in the presence of flunitrazepam, this ligand is irreversibly incorporated into a proportion (25%) of the Bz binding sites, leading to a presumed conformational change in the remaining sites. This results in a dramatically decreased affinity for Bz agonists but not antagonists [26–28] (see also Table 4). Two imidazopyridazines were tested (Table 4); as can be seen, there was virtually no decrease in their affinity for PAL Bz sites compared with control receptors.

Within the imidazopyridazine series, several compounds bearing nitro groups have been synthesized (e.g. GBLD307; see structure, Table 1). In preliminary experiments it was not possible to get these compounds to label covalently Bz receptors under UV light (same conditions as used for nitrazepam in the PAL experiments).

Interaction with type I and II sites

Several compounds tested (e.g. GBLD238) gave slopes in Hill plots significantly less than unity, suggesting a possible preferential interaction with a

Table 1. Activity and structures of some substituted imidazo[1,2-*b*]pyridazines vs [^{3}H]diazepam binding to rat brain plasma membranes



Lab. code no.	R^1	R^2	R^3	IC_{50} (nM)
GBLD 137	SCH_2Ph	OMe	Ph	25 ± 3 (3)
GBLD 150	$SCH_2C_6H_4Cl$-*o*	OMe	Ph	54 ± 12 (2)
GBLD 167	OC_6H_4OMe-*o*	OMe	Ph	70 ± 12 (2)
GBLD 168	OC_6H_4OMe-*m*	OMe	Ph	463 ± 63 (2)
GBLD 177	$SCH_2C_6H_4Me$-*p*	OMe	Ph	74 ± 8 (2)
GBLD 190	$SCH_2C_6H_4OMe$-*m*	OMe	Ph	11 ± 3 (3)
GBLD 214	$SCH_2C_6H_4OMe$-*o*	OMe	Ph	9 ± 2 (2)
GBLD 219	$SCH_2C_6H_4NO_2$-*p*	OMe	Ph	24 ± 7 (2)
GBLD 220	$SCH_2C_6H_4NH_2$-*p*	OMe	Ph	9 ± 2 (2)
GBLD 221	$OCH_2C_6H_4OMe$-*m*	OMe	Ph	6.4
GBLD 230	$SCH_2C_6H_4NH_2$-*o*	OMe	Ph	49 ± 9 (2)
GBLD 231	$NHCH_2C_6H_4OMe$-*m*	OMe	Ph	2.9 ± 1.4 (2)
GBLD 233	$SCH_2C_6H_4OMe$-*m*	OMe	C_6H_4F-*p*	11 ± 6 (2)
GBLD 251	F	OMe	C_6H_4Me-*p*	30 ± 13 (2)
GBLD 254	$NHCH_2C_6H_4OMe$-*m*	OMe	C_5H_4N-*β*	2.1 ± 0.6 (3)
GBLD 266	$SCH_2C_6H_4OMe$-*m*	OMe	C_6H_4F-*m*	10 ± 1 (2)
GBLD 268	OC_6H_4OMe-*o*	OMe	C_6H_4F-*m*	65 ± 17 (2)
GBLD 274	$NHCH_2C_6H_4OMe$-*m*	OMe	C_6H_4F-*p*	1.5 ± 0.5 (2)
GBLD 293	$SCH_2C_6H_4OMe$-*m*	OMe	$C_6H_4CF_3$-*m*	163 ± 7 (2)
GBLD 305	OC_6H_4OMe-*o*	CH_2NHAc	Ph	⩾10,000
GBLD 307	$NHCH_2C_6H_4OMe$-*m*	OMe	$C_6H_4NO_2$-*m*	2.5 ± 0.5 (3)
GBLD 308	$NHCH_2C_6H_4OMe$-*m*	OMe	C_6H_4Me-*p*	3.2 ± 0.3 (2)
GBLD 312	$NHCH_2C_6H_4OMe$-*o*	OMe	C_6H_4F-*p*	2.2 ± 1.3 (2)
GBLD 313	$NHCH_2C_6H_4OMe$-*m*	OMe	$C_6H_4NH_2$-*m*	1.8 ± 0.4 (2)
GBLD 345	$NHCH_2C_6H_4OMe$-*m*	OMe	$C_6H_4NH_2$-*p*	1.0 ± 0.1 (2)
GBLD 318	Cl	CH_2NHAc	Ph	474
GBLD 322	F	$CH_2NHCOPh$	C_6H_5Me-*p*	8.2
GBLD 331	H	$CH_2NHCOPh$	Ph	214
Diazepam				4.2 ± 0.3 (3)
CL218,872				153

All assays were conducted in the presence of 100 μM GABA.

IC_{50} values were calculated by computer-assisted log-logit analyses.

Each compound was tested at 4–5 separate concentrations in triplicate or quadruplicate; these concentrations were chosen to span the IC_{50} value estimated from an initial assay at a single screening concentration. When assays were repeated, IC_{50} values given are means ± SEM for the number of determinations in brackets. Correlation coefficients of fits to log-logit analyses were better than 0.96.

All laboratory code numbers bear the prefix, GBLD.

CL218,872 is 3-methyl-6-(3′-trifluoromethylphenyl)triazolo[4,3-*b*]pyridazine.

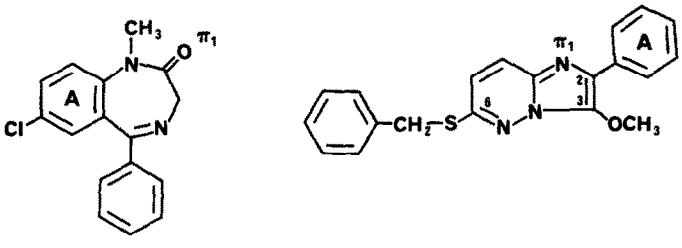


Fig. 1. Structure of diazepam and 6-benzylthio-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (GBLD 137), a representative compound of the imidazopyridazines reported in this paper. The aromatic ring (designated "A") and the spatially related proton-accepting group (designated π_1) as per the model of Bz ligands proposed by Fryer [29, 30] are indicated on the diazepam structure. The proposed equivalent sites, considered important for receptor site binding, are also given for the imidazopyridazine (see Discussion).

Table 2. Inhibition of [^{3}H]diazepam binding to central- and peripheral-type Bz binding sites

Compound	IC_{50} (nM) for inhibition of [^{3}H]diazepam binding	
	CNS membranes	Kidney membranes
Diazepam	4.3 ± 0.3 (3)	73
Clonazepam	5*	3500 ± 780 (3)
Ro5-4864	163,000*	3.6 ± 0.2 (3)
GBLD221	6.4	1080
GBLD231	2.6	1300
GBLD233	5.4	1250
GBLD251	17.4	⩾10,000
GBLD305	⩾10,000	555
GBLD318	474	177
GBLD322	8.2	168
GBLD331	214	>10,000
GBLD451	(54% at 10,000 nM)†	288
GBLD472	(50% at 10,000 nM)	366
GBLD579	(45% at 1000 nM)	158

Refer to legend to Table 1 for details.
* Published previously [36].
† Data in brackets represent per cent inhibition at the single concentration stated.
Structures of compounds not listed in Table 1 were as follows:

	R^1	R^2	R^3
GBLD451	SCH_2Ph	CH_2NMe_2	CH_2CH_2Ph
GBLD472	Cl	$CH_2NHCOMe$	CH_2CH_2Ph
GBLD579	OC_6H_4OMe-*o*	H	Ph

subset of Bz receptors (cf. CL218,872). However, no further effort was made to examine interactions of the imidazopyridazines with "classical" Bz_1 or Bz_2 sites.

Studies in vivo

Results of a study on *in vivo* Bz receptor binding in mice are given in Table 5. Clinical observations in the dosed mice are also reported. It may be noted that while the three imidazopyridazines selected caused varying degrees of sedation (but without loss-of-righting reflex), one of them (GBLD308) caused minimal receptor occupancy (at 60 min after dosing), despite having a high affinity for rat brain Bz receptors. While there could be a pharmacokinetic explanation for this, the fact that sedation was still

Table 3. Inhibition of [^{3}H]flumazenil binding: GABA shift *in vitro*

Compound	IC_{50} (nM)		Ratio
	Control	+GABA (100 μM)	
Agonists			
Diazepam*	8.5 ± 0.2 (8)	3.7 ± 0.2 (8)	2.3
Oxazepam*	46.8 ± 1.5 (8)	19.7 ± 0.6 (8)	2.4
CL218,872†	230	140	1.6
Antagonists			
Ro 15-1788	—	—	1.0
PRCC	3.4 ± 0.3 (8)	3.4 ± 0.3 (8)	0.99
MECC	3.8 ± 0.2 (8)	5.2 ± 0.2 (8)	0.73
Test compounds			
GBLD231	6.7	3.2	2.1
GBLD322	42	19	2.2
GBLD233	42	17	2.5
GBLD251	89	44	2.0

IC_{50} values are means ± SEM for the number of determinations in brackets.
* Published previously [18].
† Close to previously published values of 241 ± 5 and 146 ± 4 nM [18].
PRCC, propyl-β-carboline-3-carboxylate; MECC, methyl-β-carboline-3-carboxylate.

Table 4. Inhibition of [^{3}H]flumazenil binding in control and PAL rat brain membranes

Compound	IC_{50} (nM) Control membranes	IC_{50} (nM) PAL membranes	Ratio (PAL/control)
Agonists			
Diazepam	33.2	2201	66.3
Clonazepam*	2.53 ± 0.13	52.2 ± 6.1	20.6
Antagonists			
PRCC*	1.31 ± 0.06	0.94 ± 0.04	0.72
Ro15-1788*	2.24 ± 0.07	1.75 ± 0.08	0.78
Test compounds			
GBLD322	76	77	1.0
GBLD233	40	73	1.85

See Materials and Methods for experimental details.
Refer to the legend to Table 1 for information on calculation of IC_{50} values.
* Published previously [20].
PRCC, propyl-β-carboline-3-carboxylate.

Table 5. Binding of [^{3}H]flumazenil to mouse brain *in vivo*

Compound	Dose p.o. (mg/kg)	Displacement of binding at 60 min* (%)	Clinical observations	*In vitro* IC_{50} in rats (nM)
Flumazenil	10	90 (2)	No sedation	—
Clonazepam	10	89 (2)	Moderate–heavy sedation	—
GBLD308	20	4–15 (3)	Sedation	3.2
GBLD313	20	63 (3)	Moderate sedation	1.8
GBLD345	20	57 (2)	Heavy sedation	0.9

* Data are means for the number of mice given in brackets.
† *In vitro* data for [^{3}H]diazepam binding in rats from Table 1.
A quite high oral dose of clonazepam, a standard Bz agonist, was chosen to give an obvious pharmacological effect *in vivo*. A similar but somewhat larger dose of test compound was chosen as a single screening dose in order to maximize the likelihood of seeing an *in vivo* receptor interaction, allowing for possible pharmacokinetic barriers to CNS penetration.

apparent at 1 hr suggests the possibility that in mice the compound may be acting at another receptor site(s).

DISCUSSION

A large number of 2-phenyl (and substituted phenyl) imidazo[1,2-*b*]pyridazines have been synthesized and a significant number shown to exhibit high binding affinity to Bz binding sites on rat brain membranes. Several compounds in the series bound to peripheral-type Bz receptors (on rat kidney membranes), with some evidence of a greater selectivity for this mitochondrial site than the CNS site. It may be noted that the latter compounds carry large substituents on the 3-position of the imidazopyridazine nucleus, compared with the other compounds tested which carry a methoxy substituent at C3. Further structure–activity studies have led to the synthesis of compounds highly potent and selective at the peripheral-type site (unpublished).

The observation that GABA significantly enhanced the inhibitory potencies of selected imidazo[1,2-*b*]-pyridazines on [^{3}H]flumazenil binding suggests that these compounds are agonists at Bz receptors; this may be consistent with structure–activity predictions based on the model proposed by Fryer *et al.* [29, 30] for Bz agonists (see below).

PAL of a proportion of central Bz receptors with a suitable Bz ligand results in a dramatically decreased affinity of the remaining sites for Bz agonists but not antagonists [26–28]. Studies on the interaction of several imidazo[1,2-*b*]pyridazines with PAL Bz receptors showed that there was no significant decrease in their affinity for PAL sites as compared with control sites. However, these results cannot be taken as evidence that the compounds are acting as antagonists, since previous work [20, 31] showed that the technique was not able to distinguish between other non-Bz structures which exhibited agonist, partial agonist or antagonist profiles at Bz receptors, in contrast to results with the Bzs

themselves. Thus, the use of this *in vitro* technique which can identify Bz agonists and antagonists is not applicable across different chemical classes of ligands, i.e. the usefulness of the PAL procedure for differentiating Bz receptor agonists and antagonists appears to be confined to the Bz class of drugs and is not generally applicable to different chemical classes which have receptor binding activity. Nevertheless, it appears that the imidazo[1,2-*b*] pyridazines may bind to Bz receptors in a somewhat different manner to the "classical" Bz agonists, e.g. diazepam, and therefore there is a possibility that they may have a different pharmacological activity profile *in vivo*.

Two types of Bz receptor, Bz_1 and Bz_2, originally were proposed on the basis of pharmacology and distribution. However, with the identification of multiple isoforms of α, β and γ subunits that are thought to constitute $GABA_A$ receptors, the heterogeneity of the Bz receptor population has the potential to be very much greater than first thought [5]. Therefore, to date, no effort has been made to examine interactions of any of the imidazopyridazines with "classical" Bz_1 or Bz_2 sites.

In an *in vivo* receptor binding assay in mice using labelled flumazenil, one of three compounds tested (Table 5) had very low occupancy of central Bz receptors (i.e. gave very limited displacement of tritiated flumazenil binding) under the conditions of the assay, despite causing sedation in the mice and having high *in vitro* potency versus binding of diazepam to rat brain synaptic membranes. The other two compounds tested (also with low nanomolar IC_{50} values versus diazepam binding to brain membranes *in vitro*) had receptor occupancies of 57–63% (cf. flumazenil of near 90%).

In some related *in vivo* experiments conducted by F. Hoffmann LaRoche and Co., Basle (unpublished), compounds GBLD231 (IC_{50} vs diazepam binding in rat brain of 2.9 nM) and GBLD221 (IC_{50} of 6.4 nM) prevented pentylenetetrazole-induced convulsions in rats (ED_{50} values near 4.9 and 100 mg/kg p.o., respectively) but were inactive in mice. Similarly, GBLD231 was orally active in a Geller–Seifert type conflict test in rats (doses as low as 0.5 mg/kg) but was inactive in mice.

These data suggest the possibility of a species-specific effect of some of these compounds, but more detailed investigations are required to ascertain whether there is a pharmacological or pharmacokinetic explanation for this. Parenthetically, it may be noted that the Bz, quazepam, has been reported to be pharmacologically inactive in rats but active in other species.

A number of studies have sought to establish a common three-dimensional feature of different structures binding to Bz receptors. Based on structure–activity relationship studies on Bz and non-Bz type compounds which have IC_{50} values in the nanomolar range, Fryer [29] postulated that there are two major sites for binding of ligands at Bz receptors, namely, an aromatic or heteroaromatic ring (which he called "A"), spatially related to a proton-accepting group (designated π_1). In this model, π_1 is proposed to lie above the plane of the aromatic nucleus "A" whereas the relative distance (measured in Å) from the centre of the "A" ring to the proton-accepting group π_1 can change. Fryer related the variability of the "A" to π_1 distance to *in vivo* activity in that as this distance increased, the activity profile shifts from agonist to antagonist to inverse agonist. Thus, for "A" to π distances of 3–6.5 Å, 6.5–7.45 Å and >7.5 Å, compounds would be likely to be agonists, antagonists and inverse agonists, respectively [30].

We have proposed [32] that the 2-phenyl group on the imidazo[1,2-*b*]pyridazines may correspond to the "A" ring in Fryer's model [30] and the imidazole nitrogen atom was postulated to correspond to the π_1 region (Fig. 1). Detailed molecular analysis of compound GBLD137 (see Fig. 1 for structure) measured the distance from the centre of the 2-phenyl ring to this potential proton-accepting centre as less than 6 Å, suggesting a full receptor antagonist, according to this model.

The potential anxiolytic activity, as determined in an elevated plus-maze apparatus with rats, of several imidazo[1,2-*b*]pyridazines has been reported recently [33].

The structure of the imidazo[1,2-*b*]pyridazine compounds reported here may be compared with the nitrogen heterobicycles, CL218,872, zopiclone, tracazolate, CGS-9896 [34], EMD 39593 and EMD 41717 [35] which bind to Bz receptors; CL218,872 is a 2-*aza* derivative of the imidazo[1,2-*b*]pyridazine ring system.

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APPENDIX

Details of chemical syntheses of the compounds reported in this paper are to be found in the following references (the number following the reference is the GBLD laboratory code, see Tables 1 and 2):

[11] 137, 150, 177, 219, 220, 230;
[12] 167, 168, 190, 214, 233, 266, 268, 293;
[13] 221;
[14] 231, 254, 274, 307, 308, 312, 313, 345 (=314);
[16] 305, 318, 322, 579;
unpublished to date, 251, 331, 451, 472.